MNPD Crime Laboratory

Forensic Biology Quality Manual

Metropolitan Government of Nashville & Davidson County
Police Department

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1. Goals and Objectives

1.1. The Metropolitan Nashville Police Department Crime Laboratory (MNPD-CL) Forensic Biology Quality Assurance Program provides instruction, standards, and guidelines for best practices in the Forensic Biology Unit. By establishing and monitoring quality requirements, the quality system is designed to ensure that the Forensic Biology Unit operates within the criteria set forth in the most current revisions of ISO/IEC 17025, ANAB ISO/IEC 17025 Forensic Science Testing Laboratories Accreditation Requirements and the Federal Bureau of Investigation's (FBI) Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories, promoting valid and reliable information to the criminal justice system of Davidson County, Tennessee (See the MNPD Crime Laboratory Quality Manual Introduction).

2. Definitions

2.1. In addition to the definitions listed below, please reference the definitions in the FBI Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories and the Metropolitan Nashville Police Department Crime Laboratory (MNPD-CL) Quality Manual.

2.1.1. **Allelic drop-in**

2.1.1.1. Contamination manifested as one or two alleles.

2.1.2. **Analytical Threshold**

2.1.2.1. The minimum height requirement, determined through validation testing, at or above which detected peaks/signal can be reliably distinguished from background noise; peaks/signal at or above this threshold are generally not considered noise and are either artifacts or true alleles. Below are the laboratory's current analytical thresholds for the Promega PowerPlex Fusion 5C kit.

2.1.2.1.1. Blue dye = 127 RFU

2.1.2.1.2. Green dye = 131 RFU

2.1.2.1.3. Yellow dye = 158 RFU

2.1.2.1.4. Red dye = 153 RFU

2.1.2.1.5. Orange dye = 100 RFU

2.1.3. **Batch**

2.1.3.1. Samples associated with the same extraction blank.

2.1.4. **Batch Workbook**

2.1.4.1. Document utilized to process one or more batches of DNA samples through the DNA procedure.

2.1.5. **Deoxyribonucleic acid (DNA)**

2.1.5.1. A molecule that contains the genetic information used in the development and functioning of living organisms. DNA is found in every cell within the human body with the exception of mature red blood cells.

2.1.6. **Interpretable**

2.1.6.1. Data detected above the analytical threshold that support the determination (i.e., assumption) of the number of contributors to a DNA profile. Not all interpretable data may be used for comparison.

2.1.7. **Intimate**

2.1.7.1. An evidence sample, usually swabs, that is collected directly from the body of the contributor.

2.1.8. **Low template DNA**

2.1.8.1. An amplified sample containing a template amount of ≤ 125pg.

2.1.8.2. When a profile exhibits peaks at or around the stochastic threshold and heterozygous pairs exhibit a peak height ratio below 50%.

2.1.9. **Micro Slide Prep**

2.1.9.1. Micro Slide Prep is the process of creating a slide for the possibility of future microscopic examination of sperm. To begin this process, open the Forensic Biology Technical Procedures Manual and go to the procedures section for Microscopic Examination of Sperm KPIC Staining. Follow the procedure but stop after heat fixing the slide. The slide should then be stored per laboratory policy.

2.1.10. **Parent item**

2.1.10.1. The original evidence item submitted to the laboratory for analysis.

2.1.11. **Physical evidence**

2.1.11.1. Any object that can establish that a crime has been committed or provide a link between a crime and its victim and/or perpetrator.

2.1.12. **Preservation sampling**

2.1.12.1. Portion of a parent item of evidence that has been collected and retained with that item within the original packaging to be saved for future processing.

2.1.13. **Reference standard**

2.1.13.1. A sample, usually buccal swabs or blood, submitted as a source of DNA from a known individual to develop a DNA profile for comparison.

2.1.14. **Secondary standard**

2.1.14.1. A secondary standard is a sample submitted in lieu of a reference standard, that has been established to have come into direct contact with a single known individual. A secondary standard is not considered to be a reference standard. Since the sample is not a reference standard, no statistical weight will be applied to the comparison. If applicable, qualitative statements (as defined in the FBI QAS) can be made regarding the comparison.

2.1.15. **Single source sample**

2.1.15.1. A DNA profile consistent with a single individual; indicated by one or two peaks at each locus, except when a tri-allelic pattern is present at a locus.

2.1.16. **Subdivided evidence**

2.1.16.1. Multiple items of evidence that have subsequently been divided or a single item of evidence that has been sampled in multiple locations; the subdivided evidence will be assigned a unique identifier.

2.1.17. **Sub-item**

2.1.17.1. A sample collected from an original (parent) evidence item that is being retained for future use. Also, different pieces of evidence packaged together are considered sub-items.

2.1.18. **Temporary seal, convenience seal**

2.1.18.1. A modified seal that secures the package in a way to prevent loss of the item while the item is in the process of examination.

2.1.19. **Trace DNA**

2.1.19.1. DNA results generated from a sample in which no inference of body fluid is made and/or at least part of the profile occurs below the stochastic threshold.

2.1.20. **Uninterpretable**

2.1.20.1. The determination that DNA data cannot be interpreted (e.g., due to poor or limited data quality, data that fail to meet laboratory quality requirements). Uninterpretable data may result in inconclusive or no determinations.

2.1.21. **Violent offense / crime**

2.1.21.1. Any personal crime involving the threat of, or actual, serious bodily injury or death.

2.1.22. **Wearer DNA**

2.1.22.1. DNA recovered from a material possibly worn by an individual in question. Note: The definition may assist in extraction batch organization. This definition is not a reflection of activity and must not be relayed to customers or courts as such.

3. Quality Assurance Program

3.1. Quality System

3.1.1. The quality system of the MNPD-CL Forensic Biology Unit is documented, not only in the MNPD-CL Forensic Biology Quality Assurance Manual, but also in portions of the MNPD-CL Quality Manual (QM), MNPD-CL Safety Plan, and the Forensic Biology Technical Training and Procedures Manuals (TTM and TPM, respectively). Additional documentation referenced throughout this manual is listed in the References section.

3.2. Document Retention

3.2.1. The Forensic Biology Unit adheres to the retention policies set forth in the MNPD-CL QM.

3.3. Document Review

3.3.1. The MNPD-CL Forensic Biology QM, TTM, and TPM will be reviewed annually independent of audits. The DNA Technical Leader will direct, manage, and approve the annual reviews using the QMS.

3.4. Case File Review

3.4.1. Case files are annually reviewed and documented in the QMS. The review, as outlined by the DNA Technical Leader, is conducted independent of audits and the required reviews outlined under Section 12 of this manual.

4. Organization and Management

4.1. An overview of the organization and management of the MNPD-CL is addressed in the MNPD-CL Quality Manual. The manual describes, in general, the authorizations and responsibilities of the managerial staff and places accountability of the technical operations upon the DNA Technical Leader of the unit.

4.2. Human Resources

4.2.1. When fully staffed, the MNPD-CL Forensic Biology Unit employs one Forensic Biology Scientist Supervisor, one Forensic Biology DNA Technical Leader, eight Forensic Biology Scientists, and three Forensic Biology Technicians. When approving scheduled leave,

the supervisor should ensure that at least two Forensic Biology staff members are scheduled during regular business hours.

4.2.1.1. Managerial Staff

4.2.1.1.1. The management structure is set forth in the MNPD-CL Quality Manual. Below is the organizational structure within the MNPD-CL Forensic Biology Unit.

4.2.1.1.2. The Forensic Biology Scientist Supervisor and/or DNA Technical Leader is considered key management of the crime laboratory as set forth in the MNPD-CL Quality Manual. General charges of the Forensic Biology Scientist Supervisor are outlined in the Forensic Scientist Supervisor Job Description. The specific responsibilities and authorities the supervisor possesses as DNA Technical Leader are described in 5.2.5 of this manual.

4.2.1.2. DNA Technical Leader

4.2.1.2.1. The DNA Technical Leader is accountable for the technical operations of the Forensic Biology Unit.

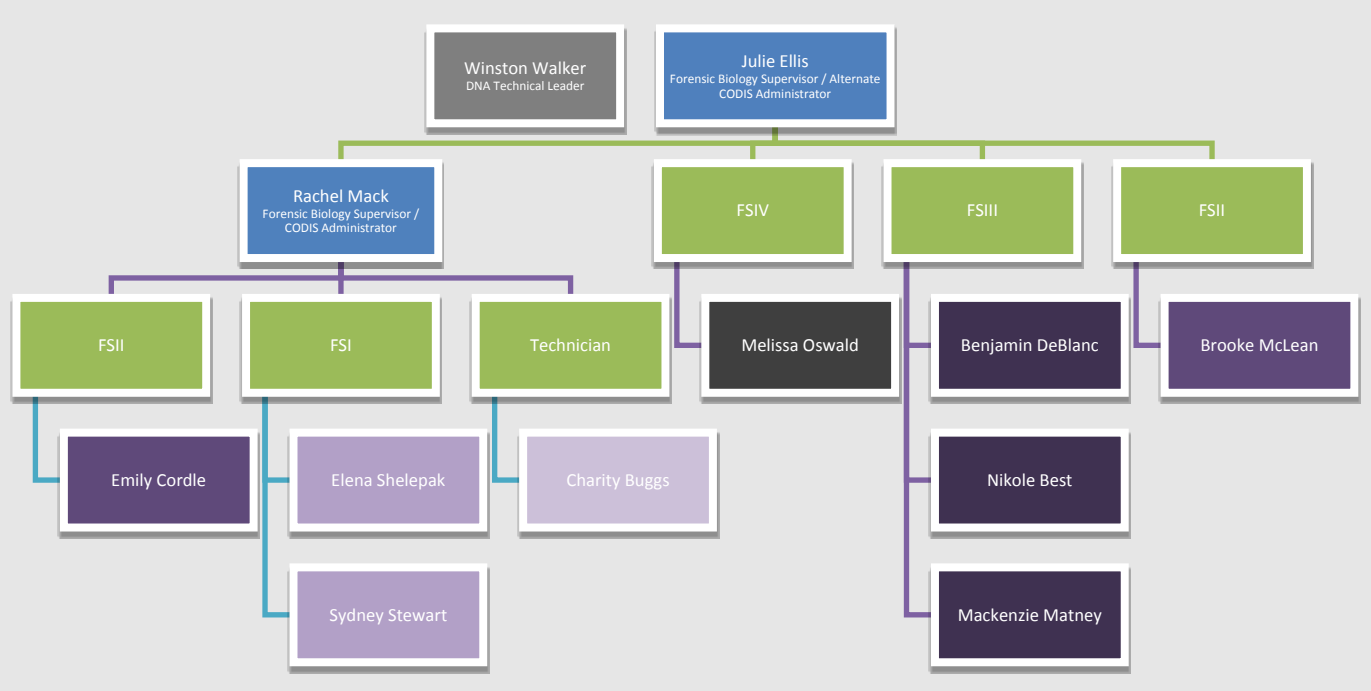
4.2.1.3. Casework CODIS Administrator

4.2.1.3.1. The specific obligations of the MNPD-CL FBU CODIS Administrator/Alternate CODIS Administrator are described in the MNPD-CL FBU CODIS Manual.

4.2.1.4. Forensic Biology Scientists

4.2.1.4.1. The MNPD-CL FBU employs at least two full-time individuals that can conduct and/or direct the analysis of forensic samples, interpret data, reach conclusions, and generate reports.

4.2.1.5. Organizational Chart: MNPD-CL Forensic Biology Organizational Chart



4.2.1.6. Contingency Plan

4.2.1.6.1. DNA Technical Leader

4.2.1.6.1.1. When the DNA Technical Leader position is vacated, a qualified analyst may be chosen to temporarily assume the position until the title is permanently filled through MNPD Human Resources.

4.2.1.6.1.1.1. If there are no qualified analysts that meet the requirements for the DNA Technical Leader, Vanessa Martinucci will temporarily assume the position until the title is permanently filled through MNPD Human Resources.

4.2.1.6.1.2. If the DNA Technical Leader position is vacated, and there is no individual who meets the requirements of the standards set forth in this document, the FBI and State CODIS Administrator will be notified immediately. The following contingency plan will be submitted to the FBI for approval:

4.2.1.6.1.2.1. A formal request may be submitted to our state DNA laboratory at the Tennessee Bureau of Investigation (TBI) requesting that all MNPD DNA cases be submitted to TBI until the vacancy is properly filled. Alternatively, DNA requests may be outsourced independently by MNPD.

4.2.1.6.1.3. Work in progress may be completed; however, no new DNA casework will be started until the contingency plan is approved.

4.2.1.6.2. Limited number of Analysts

4.2.1.6.2.1. If the number of qualified analysts falls below two, the FBI and State CODIS Administrator will be notified immediately. The following contingency plan will be submitted to the FBI within 14 days for approval:

4.2.1.6.2.1.1. A formal request may be submitted to our state DNA laboratory at the Tennessee Bureau of Investigation (TBI) requesting that all MNPD DNA cases be submitted to TBI until the vacancy is properly filled. Alternatively, DNA requests may be outsourced independently by MNPD.

4.2.1.6.2.2. Casework analyses in which DNA analytical procedures have been initiated may not be able to be completed if the number of qualified analysts falls below two full-time employees who are qualified analysts.

4.3.Applicable version of the FBI QAS

4.3.1.The version of the FBI QAS active on an employee's date of qualification (i.e., the date they are authorized to perform supervised casework to interpret and report results) will be used to assess the individual's education, experience, and training.

5. Personnel

5.1.Laboratory Personnel

5.1.1. Job Description

5.1.1.1. A job description for each member of the Forensic Biology staff is provided in the MNPD-CL Forensic Biology functional job descriptions in conjunction with the responsibilities and authorities outlined in 5.1 of this manual. For the MNPD-CL job title of each staff member, please refer to the Forensic Biology Organizational Chart.

5.1.2. Personnel Records

5.1.2.1. Records of the MNPD-CL Forensic Biology technical personnel's relevant qualifications, training, skills, and experience are documented in individual personnel files, electronically and/or physically.

5.1.2.2. Documentation to support these qualifications may be found in the individual's electronic and/or physical personnel file.

5.1.2.3. The establishment and maintenance of the personnel file is the responsibility of the individual named in the file.

5.2.DNA Technical Leader

5.2.1. Educational Requirements

5.2.1.1. The education and experience of the DNA Technical Leader is set forth in the MNPD Crime Laboratory Forensic Biology DNA Technical Leader functional job description.

5.2.2. Experience Requirements

5.2.2.1. The education and experience of the DNA Technical Leader is set forth in the MNPD Crime Laboratory Forensic Biology DNA Technical Leader functional job description.

5.2.3. Qualifications

5.2.3.1. Any DNA Technical Leader appointed on or after July 1, 2020, who is not a qualified analyst, currently or previously, in the technologies conducted by the MNPD-CL FBU will undergo training within one year of appointment to the position. The training will be sufficient to understand the scientific theory, evaluate the analysis and interpretation, and conduct troubleshooting as required by the DNA Technical Leader's responsibilities.

5.2.4. Training Requirements

5.2.4.1. In addition to the requirements described in the functional job description, for newly appointed DNA Technical Leaders, the individual must complete, or have previously completed the FBI sponsored auditor training within one year of appointment.

5.2.5. Authority and Responsibilities

5.2.5.1. Responsibilities

5.2.5.1.1. The MNPD-CL DNA Technical Leader position is held by the MNPD-CL Forensic Biology Scientist Supervisor or a qualified Forensic Scientist. The DNA Technical Leader oversees the technical operations of the Forensic Biology Unit. In addition to the responsibilities set forth in the Tasks/Work Behavior Statements of the MNPD Crime Laboratory Forensic Biology

Scientist Supervisor's functional job description (if applicable), the DNA Technical Leader is also responsible for the following:

5.2.5.1.1.1. Overseeing the technical operations of the MNPD-CL Forensic Biology Unit.

5.2.5.1.1.2. Directing and approving the review of the Forensic Biology Quality System.

5.2.5.1.1.3. Reviewing training, quality assurance, and proficiency testing programs applied to the Forensic Biology Unit.

5.2.5.1.1.4. Evaluating all validations and methods used by the Forensic Biology Unit.

5.2.5.1.1.5. Proposing new or modified analytical procedures to be used by the analyst/technician(s).

5.2.5.1.1.6. Reviewing the academic transcripts and training records of newly qualified analyst/technician/technical reviewer.

5.2.5.1.1.7. Assessing and documenting previous training when hiring new experienced analyst/technician/technical reviewer.

5.2.5.1.1.8. Ensuring that the previous training when hiring new experienced analyst/technician(s) is adequate.

5.2.5.1.1.9. Reviewing requests by contract employees for employment by multiple NDIS participating and/or vendor laboratories and ensure that no potential conflicts of interest exist.

5.2.5.1.1.10. Reviewing internal and external DNA Audit documents.

5.2.5.1.1.11. Annually reviewing the procedures of the Forensic Biology Unit.

5.2.5.2. Authority

5.2.5.2.1. The DNA Technical Leader possesses the authority to initiate, suspend and resume DNA analytical operations of the laboratory or an individual.

5.2.5.2.2. In addition, the following require the documented approval of the DNA Technical Leader:

5.2.5.2.2.1. Training, quality assurance, and proficiency testing program applied to the Forensic Biology Unit.

5.2.5.2.2.2. Qualifications of newly qualified analyst/technician/technical reviewer prior to independent casework analysis.

5.2.5.2.2.3. Modifications to the training program based on the experience of a newly hired analyst/technician.

5.2.5.2.2.4. Multimedia or internet programs used as continuing education.

5.2.5.2.2.5. Program for the annual review of scientific literature.

5.2.5.2.2.6. All validations and methods used by the Forensic Biology Unit.

5.2.5.2.2.7. Technical specifications for outsourcing agreements.

5.2.5.2.2.8. Employment of contract employees with multiple NDIS participating and/or vendor laboratories.

5.2.5.2.2.9. Corrective actions involving the Forensic Biology Unit.

5.2.6. Accessibility

5.2.6.1. The DNA Technical Leader position is a full-time position as set forth in the MNPD Crime Laboratory Forensic Biology DNA Technical Leader functional job description. The DNA Technical Leader will be accessible to the laboratory to provide onsite, telephone, or electronic consultation as needed.

5.2.7. Newly Appointed

5.2.7.1. In addition to the responsibilities outlined in 5.2.5.1, newly appointed DNA Technical Leaders will also be responsible for reviewing the following within one year of appointment:

5.2.7.1.1. Validation studies and methodologies currently being used in the laboratory.

5.2.7.1.2. Educational qualifications and training records of the currently qualified DNA analyst(s)/technician(s).

5.3.Casework CODIS Administrator/Alternate CODIS Administrator

5.3.1.The Casework CODIS Administrator position may be held by either the MNPD Crime Laboratory Forensic Biology Scientist Supervisor or an MNPD Crime Laboratory Forensic Biology Scientist. The current Casework CODIS Administrator is named in the Organizational Chart.

5.3.2. Educational Requirements

5.3.2.1. At minimum, the Casework CODIS Administrator will possess the educational requirements outlined in the MNPD Crime Laboratory Forensic Biology Scientist I functional job description.

5.3.3. Experience Requirements

5.3.3.1. The Casework CODIS Administrator may be a current or previously qualified casework DNA analyst.

5.3.4. Training Requirements

5.3.4.1. The Casework CODIS Administrator must have documented mixture interpretation training. If the individual has not previously completed the FBI auditor training, the course must be completed within one year of being appointed to the Casework CODIS Administrator position. Also, if the individual has not completed the FBI CODIS software training, this training will be completed within six months of appointment to this position.

5.3.5. Responsibilities

5.3.5.1. The CODIS Administrator/Alternate CODIS Administrator is responsible for the following:

5.3.5.1.1. Administration of the MNPD-CL local CODIS network

5.3.5.1.2. Scheduling and documenting CODIS computer training for users

5.3.5.1.3. Ensuring that the security and quality of the data stored in CODIS is in accordance with state and federal laws and NDIS operational procedures

5.3.5.1.4. Ensuring that matches within the system are dispositioned in accordance with NDIS operational procedures

5.3.5.1.5. Annual Review of the MNPD-CL CODIS Manual

5.3.5.1.6. Ensuring actions and responsibilities of CODIS Users outlined in NDIS procedures are described in the current revision of the MNPD-CL CODIS Manual

5.3.6. Authority

5.3.6.1. The CODIS Administrator/Alternate CODIS Administrator has the authority to terminate a user or the laboratory's participation in CODIS in the event an issue with the reliability or security of the data is identified.

5.3.7. Unoccupied Position

5.3.7.1. In the event the CODIS Administrator position is unoccupied, no DNA profiles will be uploaded to SDIS.

5.4.Forensic Biology Scientists

5.4.1.Routinely, an MNPD Crime Laboratory Forensic Scientist I, II, III, IV along with the MNPD Crime Laboratory Forensic Scientist Supervisor and Forensic Biology DNA Technical Leader may function as a DNA analyst. Other individuals within the MNPD-

CL organizational structure may carry out all, or part of the duties, of an analyst provided the individual possesses the required qualifications and training, in addition to successful completion of a competency test.

5.4.2. Educational Requirements

5.4.2.1. At a minimum, the DNA analyst will possess the education requirements set forth in the MNPD Crime Laboratory Forensic Scientist I functional job description.

5.4.3. Experience Requirements

5.4.3.1. An analyst will have at least six months of forensic human DNA laboratory experience gained at a facility where forensic DNA testing was performed for the identification and evaluation of biological evidence in criminal matters prior to performing independent casework. The six months may occur while participating in a formal forensic DNA training or may be six months of prior forensic human DNA lab experience. The experience must include the successful analysis of a range of samples typically associated with forensic casework.

5.5.Technical Reviewer

5.5.1. Biological Screening

5.5.1.1. An individual currently or previously qualified in biological screening may technically review biological screening casework. In the MNPD-CL Forensic Biology Unit, the technical reviewer of biological screening casework may be the Forensic Biology Technician, any level of Forensic Biology Scientist, the Forensic Biology Scientist Supervisor, or a contract employee.

5.5.2. DNA Analysis

5.5.2.1. An individual currently or previously qualified in the DNA methodologies may technically review the forensic DNA casework. In the MNPD-CL Forensic Biology Unit, the technical reviewer of DNA casework may be any level of Forensic Biology Scientist, the Forensic Biology Scientist Supervisor, or a contract employee.

5.6.Forensic Biology Technician

5.6.1.The education and experience of the Forensic Biology Technician is set forth in the MNPD Crime Laboratory Forensic Biology Technician functional job description. Based on experience and training, the MNPD-CL Forensic Biology Technician may perform biological screening and execute DNA procedures, with the exception of DNA data interpretation, reporting, and technical reviews of DNA casework. The same principles

described in 6.1, 6.1.1, and 6.1.2 of this manual, regarding training, applies to the training of the technician.

5.7. Education Review

5.7.1. The verification and approval of each analyst and technical reviewer's education by the DNA Technical Leader is documented in the QMS.

6. Training

6.1. Training Program

6.1.1. The training program for MNPD-CL Forensic Biology staff members performing casework is outlined in Module 1: Training Program Overview of the MNPD-CL Forensic Biology Technical Training Manual.

6.1.2. The MNPD-CL Forensic Biology Technical Training Manual is divided into modules designed to cover practices and technical procedures used to process Forensic Biology casework.

6.1.3. The training program for analysts hired with no experience or previous experience in a forensic human DNA laboratory is addressed below. Training requirements for analysts may vary depending on the level of experience. Regardless of experience level, the analyst will complete a competency test(s) prior to performing independent casework in the MNPD-CL Forensic Biology Unit.

6.1.4. No Previous Experience

6.1.4.1. Individuals hired with no previous experience in a forensic human DNA laboratory will complete training modules in the MNPD-CL Forensic Biology Technical Training Manual covering all Forensic Biology procedures to be performed. Successful completion of the exercises outlined in the training manual will be followed by a period of supervised casework, in which an experienced technician or analyst acts as a documented witness to the work performed by the newly trained analyst. The time spent on supervised casework will be assessed based on an evaluation of the analyst's performance. Monthly training progress updates, along with observations of performance, and test and competency test results may be used in the evaluation.

6.1.5. Previous Experience

6.1.5.1. Individuals possessing previous experience in a forensic human DNA laboratory may not be required to complete all the modules described in the

MNPD-CL Forensic Biology Technical Training Manual. Depending on the level of experience, the experienced new hire may be asked to complete portions of the module or a competency test only. Previous training will be documented in the analyst's personnel training record. The DNA Technical Leader's review of the analyst's previous training will be documented in the QMS.

6.2.Modifications to Training Program

6.2.1.For those individuals with previous training and experience in a forensic biology laboratory, the requirements described in the modules or program may be modified and/or abbreviated at the discretion of the DNA Technical Leader. The assessment and adequacy of the previous training and experience will be documented in the QMS.

6.2.2.Qualified analysts who have been on leave for a period of more than 90 days or that takes them out of a proficiency test cycle, will be required to successfully complete any applicable training missed during their absence, as well as a competency test prior to resuming casework.

6.3.Competency Testing

6.3.1.At a minimum, the experienced staff member will be required to successfully complete a competency test covering practices and procedures to the extent at which the individual will be conducting Forensic Biology casework. In addition, all applicable sections of the MNPD-CL Forensic Biology Technical Training Manual will be completed.

6.3.2.The trainee will be assessed throughout the training program using practical exercises and competency tests. Staff members should complete modules corresponding with practices and procedures for which the staff member will be responsible. Progress and completion of the various training modules are documented on the training checklists within the manual.

6.3.3.In addition to the various competencies integrated within each training module, a comprehensive practical examination will serve as a competency test following the completion of all required modules. Successful completion of the competencies is documented on the MNPD-CL Forensic Biology Technical Training Manual checklist.

6.4.Technician/Analyst Qualification for New/Additional Method

6.4.1.A competency test or a series of competency tests will be administered to personnel utilizing the newly validated method prior to its application on authentic casework samples in order to demonstrate competency.

6.4.2. The internal validation may serve as the demonstration of competency for personnel intimately involved in the validation.

6.5. Analyst Qualification for New/Additional Method for Interpretation and Reporting

6.5.1. When a new and/or additional technology, typing test kit, platform, or interpretation software is introduced, the analyst is trained and their technical skills assessed in order for the analyst to be qualified to interpret data, reach conclusions, and generate reports for casework. The analyst must successfully complete a competency test, that includes a practical component, using the new/additional technology, typing test kit, platform, or interpretation software to the extent of their participation in casework analysis.

6.5.2. For personnel intimately involved in the validation, the validation may serve as the individual's competency assessment.

6.6. Technical Reviewer

6.6.1. Individuals that perform technical reviews, but are not and/or will not be authorized as an analyst in the method, technology, typing test kit, platform or interpretation software, are required to receive training and be competency tested. The content of the training and competency test will include the case notes, data analysis, interpretation, and reporting criteria for any method, technology, typing test kit, platform, and/or interpretation software on which they were not previously qualified as an analyst.

6.6.2. For personnel intimately involved in the validation, the validation may serve as the individual's competency assessment.

6.7. Qualifications on Reinterpreting Legacy Data

6.7.1. The MNPD-CL currently does not reinterpret legacy data.

6.8. Maintenance of KSA for Reinterpretation of Legacy Data

6.8.1. The MNPD-CL currently does not reinterpret legacy data.

6.9. Training Records Review

6.9.1. The DNA Technical Leader's review of training records is documented in the QMS.

6.10. Authorities

6.10.1. Authorizations for analysts, technicians, and technical reviewers are maintained in the QMS.

6.11. Laboratory Support Personnel

6.11.1. Non-Forensic Biology Staff may be assigned to the MNPD-CL Forensic Biology Unit for support. These individuals will be trained to the extent of their job responsibilities and the training documented in the QMS.

6.12. Retraining of Personnel

6.12.1. If an analyst, technician, or technical reviewer is required to undergo retraining, the successful completion of a practical competency test, relevant to the duties upon which they were retrained, will be required. The retraining plan must be approved by the DNA Technical Leader. Documentation of the retraining will be stored as a training module in the QMS.

6.12.2. If a trainee fails the final competency test for an associated training module(s) , the Laboratory Director will determine the next course of action after consultation with the Quality Manager, Forensic Biology Scientist Supervisor, and DNA Technical Leader.

6.12.2.1. When applicable, a training plan may be created addressing the next steps for re-training, re-testing, etc., which could take into consideration factors like training history, level of experience, etc. The training plan should evaluate the root cause of the failure (i.e., is it systemic, modular, a test-taking issue, etc.) by the Laboratory Director, Quality Manager, Forensic Biology Scientist Supervisor, and DNA Technical Leader. The training plan must be approved by the DNA Technical Leader.

6.13. Training Records

6.13.1. Training and competency test records for analysts, technicians, and technical reviewers are maintained on secure MNPD-CL network drives and/or the QMS.

7. Facilities and Evidence Control

7.1.Facility Design

7.1.1.The MNPD-CL Forensic Biology Unit occupies approximately 9000 square feet of the MNPD Crime Laboratory which is located in Suite 200 on the second floor at 400 Myatt Drive, Madison, TN 37115. The unit has four office areas (rooms 2105, 2044, 2045, and 2053), an evidence storage area (room 2046), one laboratory where chemicals are prepared and supplies received (room 2052), and four laboratory areas where biological samples are processed (rooms 2047, 2048, 2049, and 2051). Below is the floor plan for the unit.

7.1.1.1.

7.1.2. Techniques performed prior to PCR amplification such as evidence examination, DNA extractions, and PCR set up are carried out in different areas and/or at different times. The examination, screening, and sampling of biological evidence are conducted in rooms 2047 and 2048. DNA extraction is performed in designated areas of room 2049, while PCR set-up is carried out in different areas of room 2049.

7.1.3. Amplified DNA product is generated, processed, and maintained in room 2051.

7.2. Laboratory Security

7.2.1. Card Readers

7.2.1.1. Four areas of the Forensic Biology Unit are controlled by card readers to prevent access by unauthorized personnel. One allows entrance into the unit. Two allow entrance into an overflow office area. The third allows access to Room 2046, the evidence storage area.

7.2.2. Video Monitoring

7.2.2.1. A video camera is positioned to allow monitoring of the evidence storage area. The camera records the field of view, when motion is detected, 24 hours a day, 365 days a year. Electronic alerts are programmed to be sent out via email. The details of this alert policy are described in the MNPD-CL QM.

7.2.3. Keys and Locks

7.2.3.1. The initial distribution of the keys and locks to the Forensic Biology Unit is documented in the Crime Laboratory Key Log. Access to certain evidence storage locations is controlled when evidence is placed in an area. When the locked storage areas are in use, the individual will document use of the evidence storage area on the Forensic Biology Key Log.

7.3.Evidence Control Program

7.3.1.The evidence control system for the MNPD-CL Forensic Biology Unit is designed to ensure the integrity of physical evidence while in the possession of personnel within the Forensic Biology Unit.

7.3.2.Items of evidence received into the Forensic Biology Unit may be collected during processing (i.e., triage or examination by another MNPD unit) or transferred from a unit within the laboratory (i.e., MNPD-CL Evidence Receiving Unit or another Forensic unit) to the Forensic Biology staff member assigned to the case.

7.3.3. Unique Identifier

7.3.3.1. Items received from the Evidence Receiving Unit (ERU) are previously marked with a unique identifier generated by the Laboratory Information Management System (LIMS).

7.3.3.2. Upon inventory, additional identifiers may need to be generated in order to accommodate items of evidence that require subdividing and/or sub-itemizing. These identifiers will also be generated from the LIMS according to the procedure outlined in Appendix A Evidence Handling of this manual and the ERU's Standard Operating Procedure (SOP).

7.3.3.3. Question and known samples received within the Forensic Biology unit will be considered evidence. Suspect standards received with a court order or MNPD 278 form are considered an Individual Characteristic Database (ICD) sample that will be treated as evidence. Preservation cuttings and/or swabs will also be considered evidence. Samples/Extracts, received or generated prior to July 1, 2020, are considered work product, for which a chain of custody is generated and tracked. Samples/Extracts generated July 1, 2020, and after are considered evidence.

7.3.3.3.1. Dilutions of the sample/extract generated as a function of the DNA analysis process are considered work product. Dilutions of the sample/extract may be discarded after use.

7.3.3.3.2. Amplification product is considered work product. Amplification product may be discarded after its associated request has undergone technical and administrative review and been released.

7.3.3.4. Samples used for testing are tracked throughout processing by labeling the tubes with the unique identifier corresponding to the source of the sample. Plates will be labeled with the six-digit date when the plate was generated, along with initials of the individual performing the procedure involving the plate (i.e., mmddyyJD). When multiple plates are run on the same day, by the same analyst, numerals will be added following the analyst's initials to distinguish the plates. Quantitation plates will be labeled with a "Q" preceding the date, while amplification plates will be labeled with an "A," and capillary electrophoresis plates will be labeled with "CE" preceding the date. The location of the sample on the plate will be distinguished through processing worksheets.

7.3.4. Chain of Custody

7.3.4.1. The chain of custody for all evidence will be routinely tracked electronically using the LIMS. Hard copy format documentation will be allowed in the event that the LIMS is not functioning. If hard copy documentation becomes necessary, analysts shall ensure that the documentation includes the signature or initials of each individual receiving and transferring the evidence, the corresponding date of the transfer, and the evidentiary items(s) transferred.

7.3.4.2. In the event that a sample is not recovered due to an instrument error, the chain of custody must be updated to accurately reflect the sample's status. Transfer the affected sample(s) in the LIMS to "Error on instrument, sample not recovered" on the same day the error occurs. If the sample(s) are not transferred on the same day, create a Chain of Custody Memorandum in QMS.

7.3.5. Control, Storage, and Preservation

7.3.5.1. Examiners taking evidence into their custody hold the responsibility to ensure that the evidence is protected from loss, cross-contamination, or deleterious change. Examiners will keep case evidence in their possession no longer than 270 calendar days without prior approval from the supervisor through a deviation request. Additional instructions are included in Appendix A Evidence Handling.

7.3.5.2. Securing Evidence and Work Product in Progress

7.3.5.2.1. General policies and practices regarding evidence control are outlined in portions of the MNPD-CL-QM and the MNPD-CL-ERU-SOP.

7.3.5.2.2. Any biological evidence shipped from the laboratory must be tracked and shipped overnight to ensure the security and preservation of the evidence.

7.3.5.2.3. Personal storage lockers, refrigerators/freezers, and caged areas are provided for the storage of evidence within the Forensic Biology Unit.

7.3.5.2.3.1. Evidence storage containers (i.e., refrigerators, freezers, lockers, etc.) are located in the Forensic Biology Evidence Storage Room and Serology (See Facility Design of this Quality Manual).

7.3.5.2.3.1.1. Typically, evidence awaiting analysis or release is stored within the Forensic Biology Evidence Storage Room.

7.3.5.2.3.1.2. Items in the process of examination may be housed in Serology or the Dark Room (See Facility Design of this Quality Manual). Cuttings/extracts will be stored in refrigerators/freezers in the Serology lab, Extraction lab, and/or the Evidence Storage room.

7.3.5.2.3.2. Evidence stored while in the possession of the Forensic Biology Unit will be secured.

7.3.5.2.3.2.1. Evidence must be secured when leaving the work area (i.e., court, lunch periods, breaks, errands, or overnight).

7.3.5.2.3.3. The use of areas or containers for secure evidence storage within the Forensic Biology Unit is documented in the MNPD-CL Key Log and/or the Forensic Biology Key Log.

7.3.5.2.3.4. Unassigned and/or overflow lockers or areas will remain accessible to all personnel until in use. Upon use of the container or area to store evidence, the examiner will take the key into his/her possession for the duration of storage. The key log shall be signed and dated once the key is in possession of the individual. Once the key is returned to the overflow locker or area, the key log will be signed and dated as being returned.

7.3.5.2.3.5. The Forensic Biology Unit Supervisor will maintain a secured lock box of spare keys to certain areas and containers. The possession of these

keys by the supervisor will be documented in the Forensic Biology Key Log.

7.3.5.2.4. Storage of biological evidence is dependent upon the condition of the item.

7.3.5.2.4.1. Liquid samples (i.e., liquid blood, semen, or oral rinses) should be stored refrigerated until the sample is prepared as a dried stain (i.e., stain card or swabs).

7.3.5.2.4.2. Dry biological evidence may be stored at room temperature.

7.3.5.3. Sealing Evidence

7.3.5.3.1. A proper seal will be administered following the examination of evidence.

7.3.5.3.2. A temporary seal may be used while evidence is in process.

7.3.5.3.3. Any seal administered must be identifiable to the person who created the seal. This requires that the seal must at least be signed or initialed and dated. The marking should cross the border of the seal and package.

7.3.5.4. *Environment*

7.3.5.4.1. To ensure the safety of laboratory staff and the quality of the work conducted in the MNPD-CL Forensic Biology Unit, certain environmental conditions will be monitored. The manner in which these conditions are monitored may differ depending on the area and/or monitoring device.

7.3.5.4.2. Hoods

7.3.5.4.2.1. The initial documentation and processing of evidence in the Forensic Biology unit is conducted within the Forensic Biology examination area (Serology or the Dark Room). Depending on the condition of the evidence (i.e., degraded or affected by decomposition), the examiner may choose to conduct initial examination in or under a hood. The use of hoods may also be required when conducting certain technical procedures such as Acid Phosphatase Mapping. The hoods will be checked annually. Documentation of the annual checks are located on the hood and in the QMS.

7.3.5.4.3. Refrigerators/Freezers

7.3.5.4.3.1. Refrigerators and freezers used to store evidence, work product, or reagents will also be monitored, at minimum once per week. The

acceptable range for refrigeration units is 2 to 8^{0}C. The acceptable range for freezers is -10 to -25^{0}C. Except during the defrosting process, if temperatures fall outside of the acceptable range during temperature check, the unit will be rechecked in approximately an hour. If the temperature remains out of range, measures will be taken to preserve the contents, and maintenance will be requested on the unit. The effect, if any, will be considered. If the conditions are found to have a possible effect on evidence, work product, and/or supplies, the incident and corrective measures will be documented.

7.3.5.4.4. Heat Blocks and Incubators

7.3.5.4.4.1. Heat blocks and incubators will be monitored when in use and documented in the case file.

7.3.5.4.5. Quality Assurance Practices

7.3.5.4.5.1. Disposable gloves and a laboratory coat will be worn when handling evidence, work product, consumables, equipment, and instruments used on biological samples. A face mask will also be worn during all the above activities except those conducted in the post amplification room. The face mask should completely cover the mouth and nose.

7.3.5.4.5.2. Change gloves frequently. Do not handle evidence or work product with gloves that have come into contact with unsterile surfaces. Change gloves between each item of evidence.

7.3.5.4.5.3. Store consumables (i.e., swabs, tubes, disposable pipettes, and slides) in closed containers.

7.3.5.4.5.4. Work with only one item of evidence at a time.

7.3.5.4.5.5. Cover bench with new covering (e.g., KimWipe, Kaydry, butcher paper, blotter, benchcote, etc.) for each item of evidence.

7.3.5.4.5.6. The handling of unknown evidence will be separated by time and/or space from the handling of standards/knowns.

7.3.5.4.5.7. Utensils used to cut, transfer, contact, etc. evidence must be sterilized prior to and after use (e.g., cleaned with 10% bleach followed by an ethanol wiping).

7.3.5.4.5.8. Quantitation and Amplification setup will be conducted in PCR enclosures and/or using a robot.

7.3.5.4.5.9. When using 96-well plates, both the location and handling of unknown samples and controls will precede reference standards and controls.

7.3.5.4.5.10. Samples suspected of containing low levels of DNA will be handled separately from samples suspected of containing high levels of DNA.

7.3.5.4.5.11. Pulse spin DNA samples (tubes, plates, etc.) prior to opening.

7.3.5.4.5.12. Analysts will use a clean tissue or decontaminate fingertips between and/or after the handling of sample tubes.

7.3.5.4.5.13. Personal protective equipment, laboratory equipment, supplies, and paperwork used in the post amplification room will not be transferred or used in other laboratory areas without decontamination.

7.3.5.4.5.14. Tube to tube, tube to plate, and plate to plate transfers shall be witnessed by another individual and documented.

7.3.5.4.5.15. Throughout the DNA process, keep samples in the order in which they were extracted with their extraction batch.

7.3.5.4.5.16. For reagents prepared in bulk, analysts/technicians will not pipette from the bulk solutions.

7.3.5.4.5.17. Individuals will not return to work in Pre-PCR and/or screening areas following work in Post-PCR within the same workday.

7.3.5.4.5.17.1. Witnessing another individual perform work in Post-PCR is not included as work in Post-PCR. However, if the witnessing individual handles any samples containing amplified DNA during the witnessing activity, they will not return to work in the Pre-PCR and/or screening areas following the witnessing activity within the same day.

7.3.5.4.6. Collection of DNA

7.3.5.4.6.1. In many instances, DNA samples (i.e., cuttings of material, swabs of possible DNA, etc.) will be collected and will proceed directly to PCR-based DNA typing. Swabs that will be immediately processed through

DNA extraction may be collected using autoclaved Ultrapure water or stain extraction buffer.

7.3.5.4.6.1.1. Note: Do not store damp or wet material (i.e., swabs, cuttings, etc.) in extraction tubes at room temperature for extended periods of time. Samples cut in extraction tubes will be stored frozen until extracted.

7.3.5.4.6.2. Samples may also be collected prior to selecting which samples to be processed for DNA. Preservation swabs, cuttings, and/or other samples collected for possible DNA testing shall be packaged, sealed, and uniquely marked, including barcode, as a sub-item of the parent item. Preservation swabs should be collected using autoclaved Ultrapure water and allowed to completely dry prior to packaging. If the sample is not tested for DNA, the sub-item will be returned with the parent item. Sub-items will be listed on the Forensic Biology Report.

7.3.5.4.6.3. For evidence containing stains that are visualized, if possible, the analyst should only collect a portion of the stain, preserving a portion of the stain on the original piece of evidence.

7.3.5.4.6.4. For swabs of trace and other suspected low level DNA samples, practices should be used in order to optimize both the recovery and extraction of the low-level samples. Examples of such practices are:

7.3.5.4.6.4.1. Minimize the amount of liquid used to prevent diluting the DNA.

7.3.5.4.6.4.2. Double swab technique (i.e., wet swab, dry swab).

7.3.5.4.6.4.3. Use swab boxes for packaging that allow swabs to be secured within the box.

7.4.Sample Consumption Policy

7.4.1.When possible, a portion of the evidence should be preserved; however, certain evidence types (e.g., trace DNA samples) and quantities may require consumption in order to produce a DNA profile that will provide inclusionary and/or exclusionary information. The analyst should use their training and discretion when assessing whether or not to consume a sample.

7.4.2.A sample will not be consumed when a request is made by the customer or the courts prior to processing.

7.5.Disposition of Evidence Policy

7.5.1.Dependent upon the request for service, the remainder of the evidence submitted to the Forensic Biology Unit will be forwarded to another Forensic Unit for further analysis or returned to the ERU upon completion and/or preservation. Transfer of the evidence to the appropriate unit will follow the procedures set forth in ERU-SOP.

7.5.2.Dispositions are communicated per the policy outlined in 7.4.1.1(A) e) of the MNPD-CL Quality Manual.

8. Validation

8.1.Method for DNA Analysis

8.1.1.The MNPD-CL Forensic Biology Unit uses methods that have been evaluated and determined to be effective and reliable for DNA analysis.

8.2.Developmental Validation

8.2.1.Internal or external developmental validations may be referenced to support the various DNA methodologies used within the unit. The developmental validation will precede the use of the method for forensic DNA analysis.

8.2.2.The following, where applicable, will be documented in the developmental validation:

- 8.2.2.1. Characterization of genetic marker
- 8.2.2.2. Species specificity
- 8.2.2.3. Sensitivity studies
- 8.2.2.4. Stability studies
- 8.2.2.5. Reproducibility
- 8.2.2.6. Case-type samples
- 8.2.2.7. Population studies
- 8.2.2.8. Mixture studies
- 8.2.2.9. Precision and accuracy studies
- 8.2.2.10. PCR-based studies including:
  - 8.2.2.10.1. Reaction conditions
  - 8.2.2.10.2. Assessment of differential and preferential amplification
  - 8.2.2.10.3. Effects of multiplexing
  - 8.2.2.10.4. Assessment of appropriate controls
  - 8.2.2.10.5. Product detection studies

8.3.Internal Validation

8.3.1. Internal validations completed for the MNPD-CL Forensic Biology's DNA processes will be reviewed and approved by the DNA Technical Leader. The internal validation will be documented in the QMS.

8.3.2. The internal validations shall be documented and summarized and will include, as applicable, the following:

8.3.2.1. Known and non-probative evidence samples or mock evidence samples

8.3.2.2. Reproducibility and precision studies

8.3.2.3. Sensitivity and stochastic studies

8.3.2.4. Mixture studies

8.3.2.5. Contamination assessment

8.3.3. Currently the MNPD-CL Forensic Biology Unit does not function as a multi-laboratory system.

8.3.4. The results from the internal validation will be used to develop the quality assurance parameters, technical procedures, interpretation guidelines, mixture interpretation guidelines, and the application of appropriate statistical calculations, when applicable.

8.3.5. If the MNPD-CL Forensic Biology Unit changes detection platforms or test kits, an internal validation will be performed according to the guidelines outlined in this manual.

8.4. Newly Validated Methods

8.4.1. Newly validated DNA methods (from amplification through characterization), typing test kit, or instrument model are checked against a certified reference material or sample traceable to a certified reference material prior to use on casework.

8.5. Modified Procedure

8.5.1. Modifications to procedures will be compared to the original procedure using DNA samples similar to those used in the validation of the original procedure. The comparison will be documented. The DNA Technical Leader will be responsible for reviewing the comparison documentation and evaluating and approving the modification prior to its application to casework.

8.6. Software

8.6.1. The laboratory uses software suitable for the intended use in the laboratory and within the limitations established during the internal validation.

8.6.2. New software, new modules of existing software, or a major revision to software that is used as a component of instrumentation, for the analysis and/or interpretation of DNA

data, or for statistical calculations shall be subject to internal validation specific to the laboratory's intended use prior to implementation in forensic DNA analysis. The evaluation and relevant validations or testing is documented in the QMS.

8.6.3. For commercial off the shelf (COTS) software products (e.g., word processing, electronic spreadsheets, database management) that the laboratory uses to create software tools (e.g., macros, workbooks, databases), the COTS software does not require a validation but, if used as a component of instrumentation, for the analysis and/or interpretation of DNA data, or for statistical calculations, the laboratory developed tool must be validated as appropriate for its intended use in the laboratory.

8.6.4. Internal Software Validations

8.6.4.1. New software or new modules of existing software that are used as a component of instrumentation, for the analysis and/or interpretation of DNA data, or for statistical calculations, used by the unit are subject to internal validations specific to the laboratory's intended use prior to the implementation in casework.

8.6.4.2. Internal software validations must include, at minimum, the following:

New software or new modules of existing software….	Functional Testing	Reliability Testing	Accuracy & Precision	Sensitivity Study	Specificity Study
…used as a component of an instrument	X	X			
…for analysis or interpretation of DNA data	X	X	As applicable	As applicable	As applicable
… for statistical calculations	X	X	As applicable		
Regression testing is also required for any major revision.					

8.6.4.3. Regression testing is required for a major revision. Examples of a major revision can include, but are not limited to, modifications of any algorithm, any statistical and/or calculation equation, sequence alignment strategy, data reports, and/or export of results.

8.6.5. A minor revision to software used as a component of instrumentation, for the analysis and/or interpretation of DNA data, or statistical calculations will require at a minimum, a functional test. Examples of a minor revision can include, but are not limited to, cosmetic modifications, improved printing or viewing features, fixing invalid error messages or a modification that only impacts a module within the software that will not be used.

8.7. Quality Records Retention

8.7.1. The policy regarding quality record retention is addressed in the MNPD-CL Quality Manual.

8.7.2. Developmental validation studies, internal validation studies, modified method evaluations, and software validation and testing, including the approval of the Technical Leader, are retained and available for review.

9. Analytical Procedures

9.1. Technical Procedures Manual

9.1.1. The MNPD-CL Forensic Biology Unit's DNA Technical Procedures are controlled using the MNPD-CL's compliance management software. The annual review and approval of the technical procedures by the unit's DNA Technical Leader is documented through the document control software. If electronic review and approval is not available, the review and approval may be documented on paper.

9.1.2. The MNPD-CL Forensic Biology Unit maintains DNA procedures for each analytical method used by the unit. The reagents and equipment used are specified in the "Equipment/Materials/Reagents" section of the procedure. Procedures for DNA sample preparation are outlined in general in Appendix A Evidence Handling of this manual and in more detail in the "Procedure" section of each technical procedure. Different extraction methods may be employed based on sample type. Each of the approved extraction methods is included in the MNPD-CL Forensic Biology Technical Procedure Manual. The controls used in each process are listed in the "Standards and Controls"

section of each technical procedure. Data interpretation is outlined in the Data Interpretation technical procedure.

9.2.Reagents

9.2.1.DNA reagents are deemed suitable based on internal validations.

9.2.2.A master list of the reagents used by the Forensic Biology Unit is located in sections 9.2.4 and 9.2.5 of this manual.

9.2.3.The procedure for the documentation of commercial reagents is outlined in Appendix B Ordering and Receiving of this manual.

9.2.4. Purchased: * denotes critical reagents and ^ denotes critical supplies which are QC'd prior to use in casework.

9.2.4.1. *Kastle-Meyer Reagent Stock Solution
9.2.4.2. *Hematrace Cards
9.2.4.3. α-Naphthyl Phosphate
9.2.4.4. ^Cytoseal
9.2.4.5. Ethanol
9.2.4.6. Fast Blue B
9.2.4.7. ^Nuclear Fast Red/Kernechtrot Solution
9.2.4.8. ^Picroindigocarmine
9.2.4.9. *p30 Kits
9.2.4.10. *EZ1/2 Investigator Kit
9.2.4.11. *Proteinase K
9.2.4.12. *Buffer G2
9.2.4.13. Buffer ATL
9.2.4.14. *Plexor HY Kit
9.2.4.15. *Fusion Kit
9.2.4.16. *HiDi Formamide
9.2.4.17. *Cathode Buffer Container
9.2.4.18. *Anode Buffer Container
9.2.4.19. *POP-4 Polymer Packs
9.2.4.20. RNAse P Plate
9.2.4.21. 7500 Spectral Calibration Kit I
9.2.4.22. Plexor Dye Calibration Kit
9.2.4.23. PowerPlex 5C Matrix Standard

9.2.4.24. Conditioning Reagent

9.2.4.25. Hydrogen Peroxide

9.2.4.26. 1M Tris, pH 8.0

9.2.4.27. 0.5M EDTA, pH 8.0

9.2.4.28. 5M NaCl

9.2.4.29. Glacial Acetic Acid

9.2.4.30. DTT

9.2.4.31. Sodium Acetate Anhydrous

9.2.4.32. *GenTegra-DNA

9.2.5. Prepared: * denotes critical reagents which are QC'd prior to use in casework.

9.2.5.1. AP Spray Solution A

9.2.5.2. AP Spray Solution B

9.2.5.3. *AP Spray Working Solution

9.2.5.4. *Buffer ATL Working Solution

9.2.5.5. *DTT

9.2.5.6. *3% Hydrogen Peroxide

9.2.5.7. *TE Buffer

9.2.5.8. *Autoclaved water

9.2.6. Reagent Preparation

9.2.6.1. At a minimum, all prepared reagents in the laboratory will be labeled with the identity of the reagent, the lot number, and the initials of the individual preparing the reagent. The lot number defines the expiration date of the reagent.

9.2.6.2. All reagents will be given an internal lot number that contains a given prefix and the expiration date of the reagent (e.g., BL013015). Expiration dates may be extended provided the validity of the reagent is tested and documented. This may be accomplished through a QC or controls.

9.2.6.3. If commercial reagents are not provided an expiration date by the manufacturer or distributor the laboratory will designate the expiration through research (e.g., vendor communication – previous or new, literature research, etc.).

9.2.6.4. General Materials/Equipment Needed

9.2.6.4.1. Graduated cylinders

9.2.6.4.2. Storage bottles

9.2.6.4.3. 15 mL conicals

9.2.6.4.4. 1.5 mL tubes

9.2.6.4.5. Pipettes and tips

9.2.6.4.6. Glass beakers

9.2.6.4.7. Stir bar

9.2.6.4.8. Stir plate

9.2.6.4.9. Serological pipettes

9.2.6.4.10. Funnel

9.2.6.5. Acid Phosphatase (AP) Solutions

9.2.6.5.1. Glacial Acetic Acid

9.2.6.5.2. Sodium Acetate, anhydrous OR Sodium Acetate trihydrate

9.2.6.5.3. Ultrapure Water

9.2.6.5.4. Sodium α-naphthyl phosphate

9.2.6.5.5. Fast Blue B

9.2.6.6. AP Spray Solution A

9.2.6.6.1. If using Sodium Acetate, Anhydrous:

9.2.6.6.1.1. Add 100 mL Ultrapure water to a glass beaker. Add stir bar and place on stir plate under fume hood. Turn stir plate on.

9.2.6.6.1.2. Add 12 g Sodium Acetate, anhydrous.

9.2.6.6.1.3. Add 10 mL glacial acetic acid.

9.2.6.6.1.4. Add 1 g Fast Blue B.

9.2.6.6.1.5. Allow to stir until fully incorporated.

9.2.6.6.2. If using Sodium Acetate trihydrate:

9.2.6.6.2.1. Add 100 mL Ultrapure water to a glass beaker. Add stir bar and place on stir plate under fume hood. Turn stir plate on.

9.2.6.6.2.2. Add 20 g Sodium Acetate trihydrate.

9.2.6.6.2.3. Add 10 mL glacial acetic acid.

9.2.6.6.2.4. Add 1 g Fast Blue B.

9.2.6.6.2.5. Allow to stir until fully incorporated.

9.2.6.6.3. Decant AP Spray Solution A into a brown bottle. Label bottle with name, internal lot number of reagent, preparer's initials, and storage requirements. Store brown bottle of AP Spray Solution A at 2-8°C up to six weeks.

9.2.6.7. AP Spray Solution B

9.2.6.7.1. Add 10 mL Ultrapure water to a glass beaker. Add stir bar and place on stir plate under fume hood. Turn stir plate on.

9.2.6.7.2. Add 0.8 g sodium α-naphthyl phosphate.

9.2.6.7.3. Allow to stir until fully incorporated.

9.2.6.7.4. Decant AP Spray Solution B into a brown bottle. Label bottle with name, internal lot number of reagent, preparer's initials, and storage requirements. Store brown bottle of AP Spray Solution B at 2-8°C for six weeks.

9.2.6.8. AP Spray Working Solution

9.2.6.8.1. Add 10 mL AP Spray Solution A to a spray bottle.

9.2.6.8.2. Add 1 mL AP Spray Solution B.

9.2.6.8.3. Bring up to 100 mL with Ultrapure water.

9.2.6.8.4. Place spray nozzle on bottle.

9.2.6.8.5. Gently shake to mix.

9.2.6.8.6. Label bottle with name, internal lot number of reagent, preparer's initials and storage requirements. Store spray bottle of AP Spray Working Solution at 2-8°C for one week.

9.2.6.9. 1X TE buffer

9.2.6.9.1. M Tris, pH 8.0

9.2.6.9.2. 500 mM EDTA, pH 8.0

9.2.6.9.3. Ultrapure Water

9.2.6.9.3.1. Add 990 mL ultrapure water to a glass storage bottle. Add stir bar and place bottle on stir plate. Turn stir plate on.

9.2.6.9.3.2. Add 10 mL 1.0 M Tris, pH 8.0.

9.2.6.9.3.3. Add 200µL 500 mM EDTA, pH 8.0.

9.2.6.9.3.4. Allow to stir until fully incorporated.

9.2.6.9.3.5. Autoclave glass bottle containing 1X TE Buffer and the bottle's screw cap to sterilize.

9.2.6.9.3.6. QC before use with casework

9.2.6.9.4. Label bottle with name, internal lot number of reagent, preparer's initials, and storage requirements. Store TE buffer at room temperature up to 1 year.

9.2.6.10. 1.0M DTT

9.2.6.10.1. Dithiothreitol (DTT)

9.2.6.10.2. Autoclaved Ultrapure Water

9.2.6.10.2.1. Add 10 mL autoclaved Ultrapure water to glass beaker. Add stir bar and place beaker on stir plate under fume hood. Turn stir plate on.

9.2.6.10.2.2. Add 1.55 g DTT.

9.2.6.10.2.3. Allow to stir until fully incorporated.

9.2.6.10.2.4. QC prior to use on casework

9.2.6.10.3. Prepare 500 µL aliquots in 1.5 mL tubes. Label rack of tubes with name, internal lot number of reagent, preparer's initials, and storage requirements. Place rack of aliquots in -20°C freezer. Store DTT aliquots frozen for 1 year.

9.2.6.11. Buffer ATL Working Solution

9.2.6.11.1. 1M Tris, pH 8.0

9.2.6.11.2. 500 mM EDTA, pH 8.0

9.2.6.11.3. 5M NaCl

9.2.6.11.4. Ultrapure Water

9.2.6.11.5. Buffer ATL

9.2.6.11.6. Note: If precipitate is present, place amount of buffer needed in glass beaker and heat on heat plate setting of approximately 2 with stirring bar until precipitate is dissolved.

9.2.6.11.7. 1L Volume

9.2.6.11.7.1. Add 640 mL Ultrapure water to a 1 L glass storage bottle. Add stir bar and place bottle on stir plate. Turn stir plate on.

9.2.6.11.7.2. Add 6.7 mL 1M Tris, pH 8.0.

9.2.6.11.7.3. Add 13.3 mL 500 mM EDTA, pH 8.0.

9.2.6.11.7.4. Add 6.7 mL 5M NaCl.

9.2.6.11.7.5. Add 333.3 mL Buffer ATL.

9.2.6.11.7.6. Allow to stir until fully incorporated.

9.2.6.11.7.7. Autoclave glass bottle containing Buffer ATL Working Solution and the bottle's screw cap to sterilize.

9.2.6.11.7.8. QC prior to use on casework

9.2.6.11.8. 500mL Volume

9.2.6.11.8.1. Add 320 mL Ultrapure water to a 500 mL glass storage bottle. Add stir bar and place bottle on stir plate. Turn stir plate on.

9.2.6.11.8.2. Add 3.4 mL 1M Tris, pH 8.0.

9.2.6.11.8.3. Add 6.6 mL 500 mM EDTA, pH 8.0.

9.2.6.11.8.4. Add 3.4 mL 5M NaCl.

9.2.6.11.8.5. Add 166.6 mL Buffer ATL.

9.2.6.11.8.6. Allow to stir until fully incorporated.

9.2.6.11.8.7. Autoclave glass bottle containing Buffer ATL Working Solution and the bottle's screw cap to sterilize.

9.2.6.11.8.8. QC prior to use on casework

9.2.6.11.9. Label bottle with name, internal lot number of reagent, preparer's initials, and storage requirements. Store Buffer ATL Working Solution at room temperature up to 1 year.

9.2.6.12. Autoclaved Ultrapure Water

9.2.6.12.1. Add Ultrapure water to a glass storage bottle (Do not fill more than 2/3 due to autoclaving)

9.2.6.12.2. Autoclave glass bottle containing water and bottle's screw cap to sterilize

9.2.6.12.3. QC prior to use on casework

9.2.6.12.4. Label bottle with name, internal lot number of reagent, preparer's initials, and storage requirements. Water may be stored up to 1 year.

9.2.6.13. 3% Hydrogen Peroxide

9.2.6.13.1. Add 10 mL of 30% Hydrogen Peroxide to a 100 mL graduated cylinder

9.2.6.13.2. Fill graduated cylinder to 100 mL mark with Ultrapure water

9.2.6.13.3. Transfer diluted Hydrogen Peroxide (3%) to plastic squirt bottle

9.2.6.13.4. QC prior to use on casework with KM QC

9.2.6.13.5. Label bottle with name, internal lot number of reagent, preparer's initials, and storage requirements. May be stored up to 1 year at 2-8°C.

9.2.6.14. Safety

9.2.6.14.1. Personal protective equipment should be worn at all times.

9.2.6.14.2. α-Naphthyl phosphate and Fast Blue B solutions emit toxic fumes when exposed to heat. Glacial acetic acid and DTT have a pungent odor and should be handled under a fume hood.

9.2.6.14.3. Caution should be used when handling these chemicals.

9.2.6.15. References

9.2.6.15.1. Reagent QC

9.2.6.15.2. MNPD-CL Forensic Biology Reagent Tracker

9.2.6.15.3. Tennessee Bureau of Investigation AP Overspray Reagents protocol

9.2.6.15.4. Florida Department of Law Enforcement 1X TE protocol

9.2.6.15.5. Mississippi Crime Lab DTT protocol

9.2.6.15.6. U.S. Army Criminal Investigation Laboratory Buffer ATL Working Solution protocol

9.3.Critical Reagents Quality Control

9.3.1.The critical reagents used by the laboratory are identified in Section 9.2 of this manual. The procedure used to evaluate these critical reagents is described below. Documentation of the evaluation is included in the QMS.

9.3.2.Each lot of the listed critical reagents/kits used by the laboratory for screening, amplifying, and genetically analyzing samples shall be quality control (QC) tested prior to use on casework. Different receipt dates of the same lot number will be differentiated and quality control tested.

9.3.3.Upon testing, if the data is determined to be unacceptable, the QC will be deemed unacceptable in the QMS workflow. Depending on the information obtained from the QC, the reagent may either be retested or deemed unusable.

9.3.4. Kastle-Meyer (Kastle-Meyer Test)

9.3.4.1. Hydrogen Peroxide 3% or 30% (dilute to 3% with Ultrapure water)

9.3.4.2. Kastle-Meyer stock solution with zinc

9.3.4.3. Ethanol

9.3.4.4. The stock bottle of Kastle-Meyer will be QC tested with a positive control (known blood sample) and a negative control (reagents only). Additionally, Hydrogen Peroxide must be QC tested each time a 3% dilution is made. QC results will be documented in the QMS. See the Phenolphthalein Test (Kastle-Meyer Test) section of the MNPD-CL Forensic Biology Technical Procedures Manual for the protocol.

9.3.4.5. Requirements for passing results: Interpretation of passing results of the Phenolphthalein (Kastle-Meyer) test can be found in the Phenolphthalein Test

(Kastle-Meyer Test) section of the MNPD-CL Forensic Biology Technical Procedures Manual.

9.3.4.6. NOTE: When aliquoting Kastle-Meyer stock solution for the Kastle-Meyer test, zinc metal shot must be added to the container to preserve the stability of the reagent.

9.3.5. Acid Phosphatase (Acid Phosphatase Test)

9.3.5.1. α-Naphthyl phosphate solution (see Reagent Preparation for preparation)

9.3.5.2. Fast Blue B solution (see Reagent Preparation for preparation)

9.3.5.3. Each prepared lot of Acid Phosphatase Working solution will be QC tested with a positive control (known semen sample) and a negative control (reagents only) on filter paper. A passing quality control test of the AP working solution will serve as a quality control test for the prepared components, AP spray solution A and AP Spray solution B, that are incorporated within the Working solution. QC results will be documented in the QMS. See the Acid Phosphatase Mapping Test section of the MNPD-CL Forensic Biology Technical Procedures Manual for the protocol.

9.3.5.4. Requirements for passing results: interpretation of passing results of the Acid Phosphatase Mapping test can be found in the Acid Phosphatase Mapping Test section of the MNPD-CL Forensic Biology Technical Procedures Manual.

9.3.6. HemaTrace Blood Test (HemaTrace)

9.3.6.1. Test device (sealed individually in a test pouch with its own desiccant and its own dropper)

9.3.6.2. HemaTrace extraction tubes containing extraction buffer (individual, pre-aliquoted)

9.3.6.3. Each lot number of HemaTrace Blood Test, including the test device and extraction buffer, will be quality control tested utilizing a positive control (known human blood) and a negative control (buffer only). The line in the control “C” area can be considered an internal procedural control. QC results will be documented in the QMS. See the HemaTrace Blood Test section of the MNPD-CL Forensic Biology Technical Procedures Manual for the protocol.

9.3.6.4. Requirements for passing results: Interpretation of passing results of the HemaTrace Blood test can be found in the HemaTrace Blood Test section of the MNPD-CL Forensic Biology Technical Procedures Manual.

9.3.7. ABAcard® p30 Test (ABAcard P30)

9.3.7.1. Test device (individually packaged)

9.3.7.2. p30 Buffer

9.3.7.3. Transfer pipette (included in kit)

9.3.7.4. Each lot number of ABAcard p30, including the test device and p30 buffer will be quality control tested utilizing a positive control (known semen standard) and a negative control (buffers only). The control line in the control "C" area can be considered an internal procedural control. QC results will be documented in the QMS. See the ABAcard p30 Test section of the MNPD-CL Forensic Biology Technical Procedures Manual for the protocol.

9.3.7.5. Requirements for passing results: Interpretation of passing results of the ABAcard p30 test can be found in the ABAcard p30 Test section of the MNPD-CL Forensic Biology Technical Procedures Manual.

9.3.8. Microscopic Examination (Micro Exam)

9.3.8.1. Nuclear Fast Red

9.3.8.2. Picroindigocarmine

9.3.8.3. Cytoseal

9.3.8.4. Each lot of Nuclear Fast Red, Picroindigocarmine, and Cytoseal will be tested against a known (non-vasectomized) semen sample under a microscope. Neat semen will be added to a Copan buccal swab for the quality control test and smeared onto a glass slide. QC results will be documented in the QMS. See the Microscopic Examination for Sperm of the MNPD-CL Forensic Biology Technical Procedures Manual for the protocol.

9.3.8.5. Requirements for passing results: Nuclear material must stain red by Nuclear Fast Red, and non-nuclear material must stain green by Picroindigocarmine for both the epithelial cells and sperm cells.

9.3.9. EZ1/2 Investigator Kit (Non-Differential Extraction or Differential Extractions)

9.3.9.1. G2 Buffer

9.3.9.2. Proteinase K

9.3.9.3. Reagent Cartridge

9.3.9.3.1. All reagent cartridges must be inspected prior to performing the QC process. The "EZ1-2 Cartridge Inspection Worksheet" must be used to document the reagent cartridge inspection. The inspection documentation

will be maintained in the QMS. Passing inspection means that all reagent cartridges match the appearance of the ground truth photo.

9.3.9.3.2. If any reagent cartridge does not pass the inspection notify the Forensic Biology Scientist Supervisors and the DNA Technical Leader to determine the next course of action.

9.3.9.4. Each lot of EZ1/2 Investigator Kit including the G2 buffer, Proteinase K and reagent cartridges within the kit will be quality control tested through either a non-differential extraction or differential extraction. Quality control tests must contain a known DNA standard as well as a reagent blank. QC results will be documented in the QMS. See the Non-Differential Extraction Procedures and Differential Extraction Procedures sections of the MNPD-CL Forensic Biology TPM for the protocol.

9.3.9.5. Note: For EZ1/2 DNA Investigator Kits, if a single component possesses more than one manufacturer lot number within a kit, the various component lot numbers will be QC'd individually.

9.3.9.6. Requirements for passing results: The typed profile results must be consistent with expected known profile, and reagent blank(s) must pass per specifications outlined in the Data Analysis section the Forensic Biology Technical Procedures Manual.

9.3.10. Plexor HY Kit (Plexor Protocols)

9.3.10.1. Master Mix

9.3.10.2. Primer Mix

9.3.10.3. DNA Standard

9.3.10.4. Amp Grade Water

9.3.10.5. Each lot number of Plexor HY Kits including the Master Mix, Primer Mix, DNA standard and Amp Grade water components will be quality control tested. A quantitative evaluation of the kit will consist of a 96-well plate set up with 2 columns of Standards from the new lot (Standards 1-7 in wells A through G, respectively) analyzed with the Plexor software. See the Quantitation section of the MNPD- CL Forensic Biology TPM for the protocol.

9.3.10.6. Requirements for passing results: The Slope and R2 of standard curve must fall within ranges indicated within the Forensic Biology Technical Procedure Manual, section 10. The Y-intercept (b) for both the autosomal and Y standard

curve must fall within the range noted in the MNPD Plexor Validation (Auto: 22.26 to 24.72; Y: 23.55 to 25.40)

9.3.11. Fusion Kit (PowerPlex Fusion)

9.3.11.1. Master Mix

9.3.11.2. Primer Mix

9.3.11.3. 2800M Control DNA

9.3.11.4. Allelic Ladder

9.3.11.5. WEN ILS 500

9.3.11.6. Each lot number of the PowerPlex Fusion Kits including the components of master mix, primer mix, 2800M control DNA, Allelic ladder and WEN ILS 500 must be quality control tested. QC will include an extracted known DNA sample and a reagent blank. Additionally, one tube of 2800M within the lot will be quantified (3) times straight by methods outlined in the Quantitation section of the MNPD-CL Forensic Biology TPM. The average quant value will be used. QC results will be documented in the QMS; in addition, the resulting quantitation value for the 2800M will be documented in the QMS workflow.

9.3.11.7. Optional: The values may also be posted on the PCR enclosures used to set-up Quant/Amplification or on the Reagent refrigerator in DNA Extraction. See the PowerPlex Fusion System section of the MNPD- CL Forensic Biology TPM for the procedure.

9.3.11.8. Requirements for passing results: The typed profile results must be consistent with expected known profile(s) and negative control(s) must pass per specifications outlined in the Data Analysis section of the Forensic Biology Technical Procedures Manual.

9.3.12. 3500 Genetic Analyzer Components (Capillary Electrophoresis)

9.3.12.1. HiDi Formamide

9.3.12.2. Cathode Buffer Container

9.3.12.3. Anode Buffer Container

9.3.12.4. POP4 Polymer

9.3.12.5. Each lot number of the various 3500 components including HiDi Formamide, Cathode buffer container, anode buffer container, and POP4 polymer must be quality control tested. A 96-well plate will be set up using the new component(s) with at least one previously passing positive and negative control. QC results will

be documented in the QMS. See the Capillary Electrophoresis section of the MNPD-CL Forensic Biology TPM for the procedure.

9.3.12.6. Requirements of passing results: Known control(s) must result in concordant typed profile(s). Passing negative control(s) must be exhibited per specifications outlined in the Data Analysis section of the Forensic Biology Technical Procedures Manual.

9.3.13. TE Buffer Solution, Proteinase K (bulk), G2 Buffer (Bulk)

9.3.13.1. TE Buffer Solution (Non-Differential through Amplification) (See Reagent Preparation for preparation)

9.3.13.2. Proteinase K

9.3.13.3. G2 Buffer

9.3.13.4. Each purchased/prepared lot of TE buffer solution, Proteinase K (bulk), and G2 buffer (bulk) will be quality control tested through a non-differential or differential extraction. The quality control test will be performed with a known DNA sample and a reagent blank. See the Non-Differential Extraction Procedures or the Differential Extraction Procedures of the MNPD-CL Forensic Biology TPM for the protocols.

9.3.13.5. Requirements of passing results: Known control(s) must result in concordant typed profile(s). Passing reagent blank(s) and negative control(s) must be exhibited per specifications outlined in the Data Analysis section of the Forensic Biology Technical Procedures Manual.

9.3.14. Autoclaved Ultrapure Water

9.3.14.1. Autoclaved Ultrapure Water (Non-Differential Extraction)

9.3.14.2. Each lot number of prepared autoclaved Ultrapure water will be quality control tested using a non-differential extraction with a known DNA sample and a reagent blank. QC results will be recorded in the QMS. See the Non-Differential Extraction Procedures section of the MNPD-CL Forensic Biology TPM for the protocol.

9.3.14.3. Requirements of passing results: Known control(s) must result in concordant typed profile(s) and reagent blank(s) must exhibit passing results per specifications outlined in the Data Analysis section of the Forensic Biology Technical Procedures Manual.

9.3.15. DTT, Buffer ATL Working Solution

9.3.15.1. DTT (Differential Extractions)

9.3.15.2. Buffer ATL Working Solution (Differential Extractions)

9.3.15.3. Each lot number of prepared DTT and Buffer ATL Working Solution should be quality control tested through a differential extraction. Quality control tests will be performed with a known DNA sample and a reagent blank. QC results will be recorded in the QMS. See the Differential Extraction Procedures of the MNPD-CL Forensic Biology TPM for the protocol.

9.3.15.4. Requirements of passing results: Known control(s) must result in concordant typed profile(s) and reagent blank(s) must exhibit passing result per specifications outlined in the Data Analysis section of the Forensic Biology Technical Procedures Manual.

9.3.16. GenTegra-DNA

9.3.16.1. Each lot number of GenTegra-DNA will be quality control tested using a non-differential extraction with a known DNA sample and a reagent blank. After extraction, and prior to quantitation, 5µl of GenTegra-DNA will be added to each extract. QC results will be recorded in the QMS. See the Non-Differential Extraction Procedures section of the MNPD-CL Forensic Biology TPM for the protocol.

9.3.16.2. Requirements of passing results: Known control(s) must result in concordant typed profile(s) and reagent blank(s) must exhibit passing results per specifications outlined in the Data Analysis section of the Forensic Biology Technical Procedures Manual.

9.3.17. Safety

9.3.17.1. Caution should be used when handling laboratory chemicals, and personal protective equipment should be used at all times.

9.3.17.2. Kastle-Meyer stock solution is a skin and eye irritant. Hydrogen Peroxide is corrosive. Keep all of the listed chemicals away from combustible material. Zinc in contact with water or damp air evolves hydrogen and the heat of reaction may ignite. Therefore, zinc should not be discarded in the wastebasket.

9.3.17.3. P30 kit reagents contain sodium azide as a preservative, which may react with lead or copper in plumbing. Upon disposal, always flush with large volumes of water to prevent build up.

9.3.17.4. Picroindigocarmine contains picric acid, which is explosive when dry. Do not allow solution to dry out.

9.3.17.5. Buffers included with the EZ1/2 Investigator Kits contain guanidine salts, which can form highly reactive compounds when combined with bleach. Do not use bleach when performing the EZ1/2 protocols or allow bleach to contact reagent waste.

9.3.17.6. The robotic arm of the QIAgility robot used in the Plexor HY and Fusion protocols moves during position calibration while the instrument lid is raised. Never click any buttons while parts of your body are within the instrument workspace.

9.3.17.7. Formamide is a known teratogen. Work should be conducted inside a safety enclosure when handling this reagent.

9.3.18. References

9.3.18.1. All below are found in the MNPD-CL Forensic Biology TPM:

9.3.18.1.1. Kastle-Meyer

9.3.18.1.2. HemaTrace Blood Test

9.3.18.1.3. Acid Phosphatase Mapping Test

9.3.18.1.4. Microscopic Examination of Sperm

9.3.18.1.5. ABAcard p30 Test

9.3.18.1.6. Non-Differential Extractions

9.3.18.1.7. Differential Extractions

9.3.18.1.8. Quantitation

9.3.18.1.9. PowerPlex Fusion

9.3.18.1.10. Capillary Electrophoresis

9.3.18.2. The following may be found in the MNPD-CL Forensic Biology QM:

9.3.18.2.1. Reagent Preparation

9.4.Quantification

9.4.1.All evidentiary samples will be quantified prior to amplification according to the Quantitation technical procedure.

9.5.Controls and Standards

9.5.1. Quantitation Standards

9.5.1.1. The use of quantitation standards is described in the Quantitation technical procedure under the “Standards and Controls” section.

9.5.2. Amplification Controls

9.5.2.1. The use of positive and negative amplification controls is described in the PowerPlex Fusion amplification technical procedures. The amplification positive and negative controls will be:

9.5.2.1.1. amplified concurrently with the sample

9.5.2.1.2. amplified on the same instrument with the samples

9.5.2.1.3. amplified using the same kit as the samples

9.5.3. Reagent Blanks

9.5.3.1. The use of reagent blanks is addressed in the "Standards and Controls" section of the extraction technical procedures (i.e., Non-Differential and Differential). Reagent blanks will be:

9.5.3.1.1. associated with each extraction batch

9.5.3.1.2. extracted concurrently with the extraction batch

9.5.3.1.3. amplified using the same testing kit, instrument model and concentration conditions as required by the sample(s) containing the least amount of DNA

9.5.3.1.4. typed using the same instrument model, injection conditions and most sensitive volume conditions of the extraction batch

9.5.4. Allelic Ladders and Internal Size Markers

9.5.4.1. The use of allelic ladders and internal size markers is addressed in the "Standards and Controls" section of the Capillary Electrophoresis technical procedure.

9.5.5. NIST Standards Performance Check

9.5.5.1. The laboratory will check its DNA procedure for the polymerase chain reaction (PCR) using a NIST Standard Reference Material (SRM) designed for PCR (i.e., 2391c or 2391d) or a standard traceable to a NIST standard. The NIST SRM is a critical reference material in which measure of traceability is applicable. The check will be conducted, at minimum, whenever substantial changes are made to the PCR procedure (i.e., changes in a test kit, platform, or software). At least two samples will be processed from amplification through characterization for each DNA typing system used in casework (i.e., PowerPlex Fusion) according to the protocols described in the MNPD-CL Forensic Biology TPM. Purchased reference material will be stored according to the vendor's recommendations. Reference material created internally will be stored in a sample refrigerator located in

Serology. The laboratory's procedures are considered to be properly functioning and calibrated to the national standard if:

9.5.5.1.1. Concordance exists between the data generated by MNPD-CL Forensic Biology Unit and the applicable data provided by NIST

9.5.5.2. All paperwork associated with the quality control test is maintained electronically within the QMS.

9.6. Interpretation of Data

9.6.1. Guidelines for the interpretation of data, including but not limited to, verification of controls and statistical and mixture interpretation, is located in the MNPD-CL Forensic Biology TPM in Data Analysis, Interpretation, and Statistics.

9.7. Formulation of Conclusions

9.7.1. The procedures used to formulate conclusions are outlined in the MNPD-CL Forensic Biology TPM under the Data Interpretation section.

9.8. Statistical Calculations and Reporting of Results

9.8.1. The procedures used to perform statistical calculations and report results are outlined in the MNPD-CL Forensic Biology TPM under the Data Interpretation and Statistical Analysis sections.

9.9. Reinterpretation of Legacy Data

9.9.1. The MNPD-CL FBU currently does not re-interpret legacy data.

9.10. Detection and Control of Contamination

9.10.1. The control, prevention, detection and monitoring of contamination is accomplished through various methods. Routine cleanings of the laboratories are documented in the QMS. The use of reagent blanks and amplification negative controls are integrated in the processing of DNA samples in the laboratory.

9.10.2. A number of detailed practices and procedures are outlined in the MNPD-CL Forensic Biology TPM and in section 7 Facilities and Evidence Control and Appendix B Ordering and Receiving of this manual.

9.10.3. Unknown Profiles

9.10.3.1. Unknown profiles generated in casework, are searched against the Reference and QC indices for quality assurance purposes. The check is documented by both the analyst and the technical reviewer on the technical review checklist.

9.10.4. Unexpected Foreign Data

9.10.4.1. An event of unexpected/foreign allelic activity will be documented in the QMS via the "Unexpected/Foreign Data Log" workflow. Unexpected data may be detected in a control and/or a sample. Unexpected/foreign data may occur as a result of a variety of reasons including, but not limited to drop-in and/or contamination.

9.10.5. Contamination

9.10.5.1. When the unexpected activity occurs at multiple loci above analytical threshold or is confirmed through re-amplification, the event will be considered contamination. If the incident is deemed contamination in the QMS workflow, a Quality System Notification is automatically generated.

9.10.5.1.1. Detecting When the Contamination was Introduced

9.10.5.1.1.1. If a contaminant is detected in a reagent blank, amplification controls, and/or a sample, an attempt will be made to determine the step at which the contaminant was introduced (i.e., evaluating quantitation data, re-transferring, re-amplifying, etc.).

9.10.5.1.1.2. If the contaminant was introduced prior to amplification, all evidence within the affected extraction batch, provided the evidence was not consumed, will be re-sampled and processed. If the evidence was consumed during initial testing, the data may be interpreted with caution under the direction of the DNA Technical Leader.

9.10.5.1.1.3. If the contaminant was introduced during amplification, provided portions of the samples with their associated controls remain, the DNA process will be repeated beginning with amplification set-up or re-sampling of the evidence. If sample(s) or their associated controls were consumed during amplification, a new sample of the evidence will be extracted. If the evidence was also consumed in the initial testing, the data may be interpreted under the direction of the DNA Technical Leader.

9.10.5.1.1.4. If the contaminant was introduced during capillary electrophoresis (CE) set-up, the samples will be retransferred from the amplification plate into a new CE plate and processed.

9.10.5.1.2. Tracing the Source of the Contamination

9.10.5.1.2.1. When an unexpected/foreign data event is deemed contamination, the source of the contamination shall be identified when possible. The contaminant profile will be compared to other profiles in the batch or batches recently processed through the laboratory.

9.10.5.1.2.2. In order to identify the source, the profile may be run through MNPD-CL's QC database, consisting of the MNPD-CL Forensic Biology Unit's Elimination Database, which includes MNPD-CL personnel, other select individuals from MNPD potentially involved in the processing of evidence, individuals entering laboratory processing areas, and unsourced profiles. Any potential hits detected in this search will be carefully evaluated based on the fullness of the contaminate profile and the relationship of the individual associated through the hit, to the evidence, laboratory, or processing of the evidence.

10. Equipment

10.1. Suitable for Methods

10.1.1. The equipment utilized to conduct forensic DNA analysis in the MNPD-CL Forensic Biology Unit was chosen based on standard methods and developmental validations conducted within the forensic community. The equipment used within the unit is identified in the QMS.

10.2. Maintenance

10.2.1. The critical instruments and equipment used in the MNPD-CL Forensic Biology unit are as follows and are identified in the QMS.

10.2.1.1. Handheld mechanical pipettes

10.2.1.2. Thermometers traceable to national or international standard(s)

10.2.1.3. Incubators/heat blocks/thermomixers used in analytical procedures

10.2.1.4. Robotic systems

10.2.1.4.1. EZ1 Advanced XL DNA Extraction Robot

10.2.1.4.2. EZ2 Connect Fx

10.2.1.4.3. QIAcube

10.2.1.4.4. QIAgility

10.2.1.5. Thermal Cyclers, including quantitative PCR

10.2.1.5.1. 7500 Real-Time PCR System

10.2.1.5.2. Veriti Thermal Cyclers

10.2.1.6. Thermal cycler temperature verification systems

10.2.1.6.1. Driftcon ProbeFixture

10.2.1.7. Genetic Analyzers

10.2.1.7.1. 3500 Genetic Analyzers

10.2.2. Below outlines the maintenance schedule and procedures for MNPD-CL FBU equipment and instruments, including those considered to be critical.

Equipment Type	Before each run	After each use	Weekly	Bi-weekly	Monthly	Quarterly	Semi-annual	Annual	As needed
Autoclave					X	X	X		X
Balances								X	X
Biological Safety Cabinets/Fume Hoods/PCR Enclosures			X					X	
Centrifuge					X				X
Combination Refrigerator/Freezer Units								X	
Driftcon® ProbeFixture™								X	X
Leeds Spectral Vision		X							X
UltraLite ALS® Turbo		X							X
Microscopes									X
M-Vac					X				X

Equipment Type	Before each run	After each use	Weekly	Bi-weekly	Monthly	Quarterly	Semi-annual	Annual	As needed
Pipettes	X	X						X	X
Qiagen EZ1 Advanced XL and EZ2 Connect Fx		X	X		X			X	
Qiagen QIAcube	X	X	X		X		X		
Qiagen QIAgility		X				X		X	X
Thermomixers			X					X	X
Veriti Thermal Cycler						X		X	
Water System									X
3500 Series Genetic Analyzer	X			X	X			X	X
7500 Real Time PCR					X		X	X	X

10.2.3. Autoclave

10.2.3.1. Monthly

10.2.3.1.1. Fill water reservoir with Ultrapure water.

10.2.3.1.2. Take out the tray holder and trays. Clean with non-bleach detergent or a non-abrasive stainless steel cleaner using a cloth or sponge. Rinse immediately with Ultrapure water to avoid staining the metal.

10.2.3.1.3. Wipe down chamber using non-bleach detergent. Rinse immediately with Ultrapure water to avoid staining the metal.

10.2.3.1.4. Clean the outer parts of the autoclave with a soft cloth and PCR Clean, or similar, followed by water.

10.2.3.2. Quarterly

10.2.3.2.1. Clean and descale the chamber, copper tubes and the reservoir using Chamber Brite. It is important that the following steps be completed without interruption.

10.2.3.2.1.1. Open the door and remove the trays from the autoclave. Spread the contents of a packet of Chamber Brite in a straight even line along the bottom of the chamber, from back to front.

10.2.3.2.1.2. Operate a sterilization cycle without dry, allowing the autoclave to reach the maximum cycle pressure in the chamber. When the cycle is finished it will automatically exhaust.

10.2.3.2.1.3. At the end of the exhaust cycle, turn the autoclave off and allow chamber to cool.

10.2.3.2.1.4. Drain the Ultrapure water from the reservoir.

10.2.3.2.1.5. Rinse and wipe the interior of the chamber with a damp cloth.

10.2.3.2.1.6. Fill the water reservoir with Ultrapure water.

10.2.3.2.1.7. Repeat a sterilization cycle without dry and without Chamber Brite powder (follow Step 3), to remove any excessive dirt in the pipes.

10.2.3.2.1.8. At the end of the exhaust cycle, turn the autoclave off and allow chamber to cool.

10.2.3.2.1.9. Drain the water from the reservoir.

10.2.3.2.1.10. Rinse and wipe the interior of the chamber with a damp cloth.

10.2.3.2.1.11. Fill the reservoir with Ultrapure water.

10.2.3.2.1.12. Press the manual water fill button and allow a small amount of water (2-4oz) to fill chamber and flush out the fill tube. Remove water from the chamber.

10.2.3.2.1.13. The instrument is ready for use.

10.2.3.3. Semi-annually

10.2.3.3.1. Clean the air jet located in the water reservoir by manipulating the air trap wire back and forth 10 times. A dirty air jet will prevent indicator strips from changing color.

10.2.3.3.2. Clean the water sensor in the rear of the chamber with a damp cloth or sponge. Cleaning the dirt off the sides of the sensor is more important than the tip.

10.2.3.3.3. Perform the steps described in the quarterly maintenance.

10.2.3.3.4. Take out the tray holder and trays. Clean with detergent or a non-abrasive stainless steel cleaner and Ultrapure water using a cloth or sponge. Rinse immediately with Ultrapure water to avoid staining the metal.

10.2.3.3.5. Clean the outer parts of the autoclave with a soft cloth and PCR Clean, or similar.

10.2.3.3.6. Clean the strainer as described below. Cleaning frequency may be reduced according to previous maintenance.

10.2.3.3.6.1. Disconnect the electrical cord and ensure there is no pressure or water in the chamber and the autoclave is cool. Open the strainer cap.

10.2.3.3.6.2. Remove the strainer element and rinse with Ultrapure water, using a brush if necessary.

10.2.3.3.6.3. Reinstall the strainer element.

10.2.3.3.6.4. Close the strainer cap.

10.2.3.3.7. Clean the door gasket with a mild detergent, Ultrapure water and a soft cloth or sponge. The gasket should be clean and smooth.

10.2.3.3.8. Clean and check the safety valve as described below.

10.2.3.3.8.1. Operate a sterilization cycle by clicking the "unwrapped Instruments Button (scissors icon), allowing a pressure of approximately 30 psi to build in the chamber.

10.2.3.3.8.2. Turn the unit off and remove the water reservoir cover.

10.2.3.3.8.3. Pull the ring of the safety valve using a tool (screwdriver) and open the safety valve for 2 sec then release. Be careful not to burn hands.

10.2.3.3.8.4. CAUTION: THIS WILL EXPOSE YOU TO HOT STEAM. DO NOT PLACE YOUR FACE OVER THE SAFETY VALVE.

10.2.3.3.8.5. Turn the unit back on and press STOP key to abort and vent the cycle.

10.2.3.3.8.6. Wait until pressure decreases to zero before opening the door.

10.2.3.4. As Needed

10.2.3.4.1. Replace door gasket.

10.2.3.4.2. Inspect the locking device for excessive wear.

10.2.3.4.3. Put a few drops of oil on the 2 door pins and door tightening bolt.

10.2.4. Balances

10.2.4.1. As Needed

10.2.4.1.1. Clean the weighing pan, draft shield and draft shield element (if present), bottom plate, and housing of the balance with a damp cloth.

10.2.4.1.2. If necessary to thoroughly clean the draft shield glass panels, remove the draft shield from the balance. When reinstalling, ensure that it is in the correct position. Follow pages 83-85 from link: http://us.mt.com/dam/LabTec/documents/NewClassic/MS/Operating_Instructions/MS-S_MS-L_BA_en_11781259E.pdf

10.2.4.2. Annually

10.2.4.2.1. Have the balances calibrated by a qualified technician.

10.2.5. Biological Safety Cabinets/Fume Hoods/PCR Enclosures

10.2.5.1. Weekly

10.2.5.1.1. Using a damp cloth, clean the exterior surfaces of the cabinet, particularly the front and top of the cabinet, to remove any accumulated dust.

10.2.5.1.2. Clean the work surface. Check the towel catch area for retained material.

10.2.5.1.3. **Biological Safety Cabinets Only:** Check the UV and fluorescent light hourmeters. When hours remaining are less than 72, lamps should be replaced. Before replacing the lamps, be sure to turn off the System Reset Switch, located on the top of the cabinet. Operate the cabinet blower, checking the percent filter life remaining. If percent filter life begins to approach 20% capacity, the cabinet filter may need replacing.

10.2.5.2. Annually

10.2.5.2.1. Have the Biological Safety Cabinets and Fume Hoods re-certified by a qualified certification technician.

10.2.5.2.2. PCR enclosure UV lamps must be replaced annually. Be sure to disconnect power before removing old lamp.

10.2.6. Centrifuge

10.2.6.1. Monthly

10.2.6.1.1. Clean and disinfect the rotor and accessories with a pH-neutral solution (e.g., PCR Clean, or similar) by removing the rotor. Clean and

disinfect the rotor bores and rotor lids with a pH-neutral solution (e.g., PCR Clean, or similar).

10.2.6.1.2. Grease rotor seals.

10.2.6.2. As Needed

10.2.6.2.1. Remove dust deposits from the ventilation slits of the centrifuge using a brush or swab.

10.2.6.2.2. Regularly check the gas spring of the centrifuge lid for proper functioning.

10.2.7. Combination Refrigerator/Freezer Units

10.2.7.1. Annually

10.2.7.1.1. Inspect the condensate evaporator pan. If needed, clean the condensate evaporator pan by sponging clean with soapy Ultrapure water. Disconnect power from the refrigerator and then unplug condensate vaporizer before cleaning.

10.2.7.1.1.1. WARNING: Evaporator pan should be allowed to cool before touching.

10.2.8. Driftcon® ProbeFixture™ using Software v2.2.0.0

10.2.8.1. As Needed/When Used

10.2.8.1.1. Clean the outside of the Driftcon® hardware and the top cover of the ProbeFixture™ with a damp, soft cloth or tissue. If dirty, the probes may be cleaned very carefully with a tissue. DO NOT APPLY ANY FORCE.

10.2.8.2. Annually

10.2.8.2.1. A performance check will be performed on the Driftcon® ProbeFixture™ and hardware box by a qualified service technician every 12 months. The vendor will perform the calibration by checking all probes at various temperatures.

10.2.9. Leeds Spectral Vision (LSV) using System Software v4.4

10.2.9.1. After Use

10.2.9.1.1. The LSV should be cleaned with diluted bleach and Ultrapure water solution or other laboratory detergent cleaner sprayed onto a soft cloth. DO NOT clean inside LSV head or inside vent holes with cleaning solution.

10.2.10. UltraLite ALS® Turbo

10.2.10.1. After Use

10.2.10.1.1. The UltraLite ALS® Turbo and its accessories should be cleaned using a soft cloth dampened with a mild detergent solution.

10.2.10.2. As Needed

10.2.10.2.1. Use a cotton ball or swab and a mild glass cleaner to remove dust and dirt from the Head Assembly lens. Avoid touching the lens.

10.2.11. Microscopes

10.2.11.1. As Needed

10.2.11.1.1. Dust and loose dirt particles can be removed with a soft brush or lint-free cotton cloth. Clinging dirt can be cleaned with a little soapy Ultrapure water or alcohol.

10.2.11.1.2. If glass surfaces, and particularly objectives, need to be cleaned, follow the information in Cleaning of Microscope Optics

10.2.11.1.3. Perform Koehler Illumination to optimize optics.

10.2.12. M-Vac

10.2.12.1. Monthly

10.2.12.1.1. M-Vac Function Test

10.2.12.1.1.1. Plug the M-Vac into a power source.

10.2.12.1.1.2. Check the filter to make sure there is no water inside (filter can be seen through the glass on the back of the machine).

10.2.12.1.1.3. Flip on power switch and verify power light comes on.

10.2.12.1.1.4. Make sure the solution bag door is closed and turn on solution pressure switch (never leave the door open). Turn off switch after bag starts to inflate.

10.2.12.1.1.5. Flip on the vacuum switch and place finger over hose connection area and check the pressure gauge changes.

10.2.12.2. As needed

10.2.12.2.1. Per vendor communication QMS WF 101588 the annual maintenance will be performed on the M-Vac by a qualified service technician once every 48 months, or as needed.

10.2.13. Pipettes

10.2.13.1. Before/After Each Use

10.2.13.1.1. Clean the exterior surfaces of the pipettes with a mild cleaning solution or suitable DNA decontamination agent.

10.2.13.2. Annually

10.2.13.2.1. The pipettes will be calibrated annually by a certified company or representative.

10.2.13.3. As Needed

10.2.13.3.1. To remove heavy contamination from liquid penetration, disassemble the lower part of the pipette by pressing the ejector and removing the ejector sleeve. Clean the ejector sleeve and exposed lower parts of the pipette and rinse with autoclaved Ultrapure water.

10.2.14. Qiagen EZ1 Advanced XL and EZ2 Connect Fx

10.2.14.1. CAUTION: Do not use bleach when cleaning the EZ1/2. Bleach may react with residual guanidinium to form chlorine gas. Use 70% - 100% ethanol and autoclaved Ultrapure water. Do not use alcohol or alcohol-based solutions to clean the EZ1/2 door. Do not use alcohol, alcohol-based solutions, or bleach to clean the EZ2 touchscreen.

10.2.14.2. After Each Use

10.2.14.2.1. Clean the worktable, tray, piercing unit, and cartridge rack with a lint free tissue moistened with 70% - 100% ethanol and then dry with a lint free tissue. Run the UV lamp for 20-30 min (optional).

10.2.14.2.1.1. EZ1 Advanced XL

10.2.14.2.1.1.1. To access the piercing unit, press “2” from the home screen. Then, press “3” from the manual operation screen. Press “Start”.

10.2.14.2.1.2. EZ2 Connect Fx

10.2.14.2.1.2.1. The instrument will prompt the analyst after the run to clean the piercing unit. Follow the on-screen instructions to lower the piercing unit. After cleaning, check the box that verifies the cleaning has been completed, then select “finish”.

10.2.14.2.2. If the instrument has been on for more than 8 hours, turn off the instrument, reboot the instrument, and then perform the UV decontamination.

10.2.14.3. Weekly

10.2.14.3.1. EZ1 Advanced XL only

10.2.14.3.1.1. Apply a small amount of silicon grease to the surface of the O-rings.

10.2.14.3.2. Clean the outside of the EZ1/2.

10.2.14.3.2.1. The EZ1 door may be cleaned with autoclaved Ultrapure water, while the screen may be cleaned with 70% - 100% ethanol.

10.2.14.3.2.2. The EZ2 door and touchscreen may be cleaned with autoclaved Ultrapure water.

10.2.14.3.2.3. Run the UV lamp for 20-30 min (optional).

10.2.14.4. Monthly – EZ2 Connect Fx only

10.2.14.4.1. Apply a small amount of silicon grease to the surface of the O-rings.

10.2.14.4.2. Run the UV lamp for 20-30 min (optional).

10.2.14.5. Annually

10.2.14.5.1. A qualified representative will perform the annual maintenance. The instrument will be performance checked prior to use on casework after the annual maintenance has been completed.

10.2.15. Qiagen QIAcube

10.2.15.1. Only use pH neutral solutions when cleaning the QIAcube. Solutions with a non-neutral pH may damage the internal components of the instrument. Also, take care that no liquid runs down the touchscreen as this may cause damage. Do not use alcohol or alcohol-based solutions to clean the QIAcube door.

10.2.15.2. Before Each Use

10.2.15.2.1. Clean the worktable, shaker rack, labware tray, outside of reagent bottle, and inside surfaces of instrument with a lint free tissue or paper towel moistened with a pH neutral solution (e.g., PCR Clean, or similar) and then with autoclaved Ultrapure water. Do not spray surfaces directly with cleaner. Clean tip adapter with autoclaved Ultrapure water only.

10.2.15.2.2. Remove the waste drawer and labware tray from the instrument. Clean the optical sensor and the gripper unit (the gripper, the stabilizing rod, and the spin column lid holder) with a lint free tissue moistened with autoclaved Ultrapure water.

10.2.15.2.2.1. To access the modules within the robotic arm, press TOOLS in the main menu. Then, select MAINTENANCE. Next, select CLEANING POSITION. Finally, select START. Follow the instructions displayed in the touchscreen.

10.2.15.3. After Each Use

10.2.15.3.1. Clean the worktable, shaker rack, labware tray, outside of reagent bottle, and inside surfaces of instrument with a lint free tissue or paper towel moistened with a pH neutral solution (e.g., PCR Clean, or similar) and then with autoclaved Ultrapure water.

10.2.15.3.2. Empty the waste drawer.

10.2.15.3.3. Additionally, clean the liner of the waste drawer with a pH neutral solution followed by autoclaved Ultrapure water.

10.2.15.4. Weekly

10.2.15.4.1. Clean the outside of the QIAcube. The QIAcube door may be cleaned with autoclaved Ultrapure water, while the touchscreen may be cleaned with 70% - 100% ethanol.

10.2.15.5. Monthly

10.2.15.5.1. Change the tip adapter ring according to the instructions found on the www.qiagen.com website (Search: change tip adapter ring. Find the instructions under the Knowledge & Support tab).

10.2.15.5.2. Perform the tightness test, which is used to check whether the tightness of the pipetting system, including the attached pipetting tip, is sufficient.

10.2.15.5.2.1. Load an empty 2 ml safe-lock microcentrifuge tube in position 1 of the shaker.

10.2.15.5.2.2. Fill a reagent bottle with approximately 96% ethanol and place in position 1 of the reagent bottle rack.

10.2.15.5.2.3. Load a tip rack of the filter tips you want to test (1000µL or 1000µL wide-bore) onto the QIAcube.

10.2.15.5.2.4. In the main menu, press TOOLS.

10.2.15.5.2.5. Select MAINTENANCE.

10.2.15.5.2.6. Select TIGHTNESS TEST.

10.2.15.5.2.7. Select the appropriate type of filter-tips (1000µL TIPS or 1000µL WIDE-BORE TIPS).

10.2.15.5.2.8. Follow the instructions displayed in the touchscreen, and press START to start the tightness test.

10.2.15.5.2.9. After the load check, the robotic arm will pick up a tip, aspirate ethanol, and move to the tube. The tip will remain in place above the tube for 2 minutes. The tip will be detached.

10.2.15.5.2.10. After the protocol is completed, open the QIAcube door and check if the tube contains liquid. If the tube is still empty and dry, the tightness of the pipetting system is adequate. If you find liquid in the tube, contact Qiagen Technical Services.

10.2.15.6. Semi-Annually/Annual

10.2.15.6.1. Change the tip adapter ring according to the instructions found on the www.qiagen.com website (Search: change tip adapter ring. Find the instructions under the Knowledge & Support tab).

10.2.15.6.2. Clean centrifuge rotor and buckets as described below.

10.2.15.6.2.1. Remove the buckets from the rotor. Undo the rotor nut on top of the rotor using the rotor key, and carefully lift the rotor off the rotor shaft.

10.2.15.6.2.2. Submerge the rotor, buckets, and rotor nut in cleaning agent. Incubate for approximately 10 minutes.

10.2.15.6.2.3. Rinse thoroughly with autoclaved Ultrapure water. Use a brush (i.e., a toothbrush or tube brush) to clean any parts that are difficult to access, such as the bucket mount and the rotor head. Wipe surfaces dry with a soft lint-free cloth. If available, dry the buckets and rotor with pressurized air. Clean the centrifuge, centrifuge rotor, and buckets with a lint free tissue or paper towel moistened with a pH neutral solution and then with autoclaved Ultrapure water. Do not spray surfaces directly with cleaner.

10.2.15.6.2.4. Apply a few drops of mineral oil on a soft, lint-free cloth, and wipe the bucket mount and rotor claw. A thin, invisible oil film should cover the bucket mount and rotor claw, but no droplets or smear should be apparent. Important: Before applying oil to the rotor buckets on the rotor, make sure that the rotor and all buckets are completely dry.

10.2.15.6.2.5. Clean the inside of the centrifuge and the centrifuge gasket and wipe dry with lint-free paper towels. Important: Make sure the gaskets remain in the proper positions.

10.2.15.6.2.6. Clean the centrifuge lid and wipe dry with paper towels.

10.2.15.6.2.7. Check the centrifuge gasket for damage. If the gasket is damaged or shows signs of wear, contact Qiagen Technical Services.

10.2.15.6.2.8. Perform the tightness test, which is used to check whether the tightness of the pipetting system, including the attached pipetting tip, is sufficient.

10.2.15.6.2.8.1. Load an empty 2 ml safe-lock microcentrifuge tube in position 1 of the shaker.

10.2.15.6.2.8.2. Fill a reagent bottle with approximately 96% ethanol and place in position 1 of the reagent bottle rack.

10.2.15.6.2.8.3. Load a tip rack of the filter tips you want to test (1000µL or 1000µL wide-bore) onto the QIAcube.

10.2.15.6.2.8.4. In the main menu, press TOOLS.

10.2.15.6.2.8.5. Select MAINTENANCE.

10.2.15.6.2.8.6. Select TIGHTNESS TEST.

10.2.15.6.2.8.7. Select the appropriate type of filter-tips (1000µL TIPS or 1000µL WIDE-BORE TIPS).

10.2.15.6.2.8.8. Follow the instructions displayed in the touchscreen, and press START to start the tightness test.

10.2.15.6.2.8.9. After the load check, the robotic arm will pick up a tip, aspirate ethanol, and move to the tube. The tip will remain in place above the tube for 2 minutes. The tip will be detached.

10.2.15.6.2.8.10. After the protocol is completed, open the QIAcube door and check if the tube contains liquid. If the tube is still empty and dry, the tightness of the pipetting system is adequate. If you find liquid in the tube, contact Qiagen Technical Services.

10.2.15.6.3. Annual maintenance may be performed by a qualified vendor.

10.2.15.6.4. A qualified representative will perform the annual maintenance and may complete some of the tasks listed above. The instrument will be performance checked prior to use on casework after the annual maintenance has been completed.

10.2.16. QIAgility using Operating Software v4.17.1

10.2.16.1. As Needed

10.2.16.1.1. When the user starts a new lot number of tips, it is recommended that the tip offsets be calibrated due to small variations that sometimes exist between the collar size of the 50µL and 200µL tips.

10.2.16.1.2. Calibrate Tip Offsets:

10.2.16.1.2.1. Ensure that a plate with Reaction or Mix function, with well sizes large enough to accommodate 50µL and 200µL tips, is available. This plate must be position and height calibrated.

10.2.16.1.2.2. Place a tube in the first well of the reaction plate.

10.2.16.1.2.3. Ensure that 50µL and 200µL tips are available, that Tip Rack Holders are calibrated and that correct tips are set as available.

10.2.16.1.2.4. Select Options/Robot Setup/Calibrate tip offsets.

10.2.16.1.2.5. In the dialog box, select the correct plate function of the plate in which tip offset calibration will be performed.

10.2.16.1.2.6. Click on the button to open the Plate/well Selector screen. Left-click on a plate to select the location of the plate in which tip offset calibration will be performed.

10.2.16.1.2.7. Ensure that the correct tip type is selected in the Select tip types to profile panel.

10.2.16.1.2.8. Define the number of tips to be used to calibrate the average offset.

10.2.16.1.2.9. Click on the Start button to start tip offset calibration.

10.2.16.2. After Use

10.2.16.2.1. Clean the worktable inside the instrument with a soft cloth sprayed with a mild pH neutral cleaning reagent.

10.2.16.2.1.1. NOTE: Do NOT wipe the rails supporting the pipetting head. Wiping the rails will remove the grease and make them more susceptible to rust.

10.2.16.2.2. Operate the UV lamp after each use of the QIAgility. Remove all worktable accessories and close the instrument lid. Click on the Light Bulb icon on the toolbar and in the dialog box ensure the exposure time is set to 15 minutes before clicking START.

10.2.16.3. Quarterly

10.2.16.3.1. The QIAgility should be decontaminated to remove DNA.

10.2.16.3.1.1. Remove all loading blocks and the tip ejector from the worktable. Wash these in detergent and rinse with clean autoclaved Ultrapure water.

10.2.16.3.1.2. Soak these components in a pH neutral cleaning agent for 15 to 30 minutes.

10.2.16.3.1.3. Thoroughly rinse the components in autoclaved Ultrapure water.

10.2.16.3.1.4. Briefly rinse with absolute ethanol and dry with a soft paper towel.

10.2.16.3.1.5. Moisten the instrument worktable with a pH neutral cleaning agent and allow to stand for 15 to 30 min. Wipe dry with clean paper towels.

10.2.16.3.1.6. Moisten the worktable with autoclaved Ultrapure water and wipe dry. Repeat this 3 times.

10.2.16.3.1.7. Moisten the worktable with a pH neutral cleaning agent and allow to air dry.

10.2.16.3.1.8. Return all components to the worktable and close the hood.

10.2.16.3.1.9. Switch on the UV lamp for 15min.

10.2.16.4. Annually

10.2.16.4.1. The pipettor should be serviced once per year by a qualified service technician.

10.2.17. Thermomixers

10.2.17.1. Weekly

10.2.17.1.1. Clean the housing and the exchangeable thermoblocks with a mild, soap based solution and a lint-free cloth. Wipe with autoclaved Ultrapure water and dry all cleaned parts.

10.2.17.2. As Needed

10.2.17.2.1. Clean the exchangeable thermoblocks immediately if sample fluid enters the bore holes or the surface.

10.2.17.3. Annually

10.2.17.3.1. Calibrate the temperature setting on each thermomixer using a NIST traceable thermometer.

10.2.18. Veriti Thermal Cycler

10.2.18.1. Quarterly

10.2.18.1.1. Clean the sample wells and heated cover with a cotton swab or cloth moistened with isopropanol, followed by a dry cotton swab or cloth.

10.2.18.2. Annually

10.2.18.2.1. Calibrate the temperature utilizing the Driftcon, according to the instructions outlined in Appendix E of this manual.

10.2.19. Water System

10.2.19.1. Millipore Direct-Q 3 UV System

10.2.19.1.1. As Needed

10.2.19.1.1.1. Cleaning the screen filter:

10.2.19.1.1.1.1. When alerted by the Millipore system, close the Feedwater Supply Valve and open the front cover.

10.2.19.1.1.1.2. Locate the Screen Filter where the Feedwater 8mm OD Tubing originates.

10.2.19.1.1.1.3. Unscrew the collar that holds the tubing to the barbed end of the fittings. Pull the tubing off of the fitting.

10.2.19.1.1.1.4. Unscrew the Screen Filter from the Feedwater pipe.

10.2.19.1.1.1.5. Flush tap water backwards through the Screen Filter.

10.2.19.1.1.1.6. Screw the Screen Filter back onto the Feedwater Supply Pipe.

10.2.19.1.1.1.7. Attach the Feedwater Tubing back onto the barbed fitting.

10.2.19.1.1.1.8. Open the Feedwater Supply Valve.

10.2.19.1.1.1.9. Close the front cover.

10.2.19.1.1.2. Replace the UV lamp:

10.2.19.1.1.2.1. The red UV Lamp Alarm will be blinking on the Display when it is time to exchange the UV lamp.

10.2.19.1.1.2.2. Open the front cover to let the system go into STANDBY mode. Unplug the electrical cord to power OFF the system.

10.2.19.1.1.2.3. Remove the SmartPak cartridge.

10.2.19.1.1.2.4. Detach the Velcro belt of the UV housing. Pull the UV housing out so that the UV lamp cable is accessible.

10.2.19.1.1.2.4.1. Use the gloves supplied with the UV replacement kit.

10.2.19.1.1.2.5. Unplug the electrical cable from the UV lamp.

10.2.19.1.1.2.6. Pull the UV lamp out of the UV housing. Ensure that you use the gloves supplied with the UV replacement kit.

10.2.19.1.1.2.7. Carefully insert the UV lamp into the UV housing.

10.2.19.1.1.2.8. Plug the electrical cable to the new UV lamp.

10.2.19.1.1.2.9. Attach the UV housing with the Velcro belt.

10.2.19.1.1.2.10. Install the SmartPak cartridge.

10.2.19.1.1.2.10.1. If the SmartPak cartridge is not being replaced, then reinstall the old SmartPak cartridge BEFORE powering ON the system. Otherwise, the system will go into FLUSH mode for 15 minutes during which no Product Water will be available.

10.2.19.1.1.2.11. Close the front cover and plug the electrical cord to power ON the system.

10.2.19.1.1.2.12. Reset the UV Timer by pressing the MAIN and + button together to enter the menu. The Display will show CO1.

10.2.19.1.1.2.13. Press the MAIN button 4 times. The Display will show CO5 and "0" days left on the UV timer.

10.2.19.1.1.2.14. Press the + and – buttons together. This will reset the UV timer to 500 days. The UV Timer has been reset.

10.2.19.1.1.3. Replace the SmartPak Cartridge:

10.2.19.1.1.3.1. Remove the protective caps from the new SmartPak. Wet the O-rings located on the ports with Ultrapure water.

10.2.19.1.1.3.2. Drain water from the Millipore. Be sure to leave the Tank Outlet Valve open after draining water.

10.2.19.1.1.3.3. Open the front cover and wait for the system to depressurize.

10.2.19.1.1.3.4. Press your thumbs on the system and swing the pack towards you to remove the pack from the system.

10.2.19.1.1.3.5. Install the new SmartPak cartridge until it is fully seated into the system ports and close the front cover.

10.2.19.1.1.3.5.1. NOTE: The Tank Outlet Valve should be left open.

10.2.19.1.1.3.6. The system will now go into FLUSH mode for 15 minutes to empty the SmartPak of air and hydrate the material inside.

10.2.19.1.1.3.7. When FLUSH mode is finished, the system will go into FILLING TANK mode automatically.

10.2.19.1.1.3.7.1. Let FILLING TANK mode run for a minimum of 2hrs with the Tank Outlet Valve open and placed to the drain.

10.2.19.1.1.3.7.2. It is recommended to leave the system in FILLING TANK overnight to ensure complete rinsing of the RO membrane.

10.2.19.1.1.3.8. Close the Tank Outlet Valve and connect it back to the system if needed. When it is full, the Display will indicate a full tank.

10.2.19.1.1.3.9. Dispense about 1L of water to ensure all air is purged out of system.

10.2.19.1.1.3.10. A new Vent Filter and Final Filter now needs to be installed.

10.2.19.1.1.4. Replacing Vent Filter (when SmartPak installed): Remove the vent filter and insert the new vent filter into the fitting.

10.2.19.1.1.5. Replacing the Final filter: Screw filter into the fitting allowing ultra pure water to fill up before shutting the white valve the final filter**.**

10.2.20. 3500 Series Genetic Analyzer using 3500 Data Collection Software v4.0.1

10.2.20.1. Before Each Run

10.2.20.1.1. If the instrument has not been restarted for the day, restart the computer and instrument. Refer to the TPM for proper order.

10.2.20.1.2. Check the status of the anode buffer container, cathode buffer container, and polymer on the dashboard. Ensure that enough of each reagent is present to complete the necessary amount of injections. Replace/replenish the reagent(s) if necessary. Visually examine the fluid level inside the anode and cathode buffer containers to ensure it is even with the fill line. Replace buffer(s) if necessary.

10.2.20.1.3. Also, examine the pump block and channels to ensure no bubbles are present. Perform the Bubble Remove Wizard if necessary. If the instrument has not been used in the past 9 days, proceed to the biweekly section.

10.2.20.1.4. Check the loading end header of the capillary array to ensure the array tips are not bent, crushed, or coated with dried polymer. Replace the array or wipe the array tips with a lint free tissue if necessary.

10.2.20.2. As needed

10.2.20.2.1. Polymer

10.2.20.2.1.1. Replace the polymer at least every 14 days or expiration date of pouch.

10.2.20.2.1.2. Restart the computer and instrument. Run the "Wash Pump and Channels" wizard. An empty anode buffer container is needed to run this wizard, so do not add new anode buffer before doing the wash.

10.2.20.2.1.3. From the Maintenance Wizards screen, select the "Wash Pump and Channels" wizard. Follow the prompts in the wizard.

10.2.20.2.2. Anode and Cathode Buffer

10.2.20.2.2.1. Replace the anode and cathode buffer at least every 14 days.

10.2.20.2.2.2. When the Anode buffer is replaced, clean the anode buffer container valve pin with a lint free tissue.

10.2.20.2.3. Capillary Array

10.2.20.2.3.1. Change the capillary array. The capillary array shall not exceed 120 injections.

10.2.20.2.3.2. From the Maintenance Wizards screen, select the INSTALL CAPILLARY ARRAY wizard. Follow the prompts in the wizard.

10.2.20.2.4. Spatial Calibration

10.2.20.2.4.1. When the capillary array is changed, a new spatial calibration must be performed.

10.2.20.2.4.2. Access the Spatial Calibration screen: Select MAINTENANCE, then select SPATIAL CALIBRATION in the navigation pane.

10.2.20.2.4.3. Select NO FILL or select FILL to fill the array with polymer before starting the calibration.

10.2.20.2.4.4. Click START CALIBRATION.

10.2.20.2.4.5. Evaluate the spatial calibration profile to ensure that you see:

10.2.20.2.4.5.1. One sharp peak for each capillary. Small shoulders are acceptable.

10.2.20.2.4.5.2. One marker (+) at the apex of every peak. No off-apex markers.

10.2.20.2.4.5.3. An even peak profile (all peaks about the same height).

10.2.20.2.4.6. If the results meet the criteria above, click ACCEPT RESULTS.

10.2.20.2.4.7. If the results do not meet the criteria above, click REJECT RESULTS, then repeat the spatial calibration. If the results still do not meet the criteria above, reposition or replace the capillary array. If issues still arise, consult the DNA Technical Leader and/or a technical representative.

10.2.20.2.5. Spectral Calibration

10.2.20.2.5.1. A spectral calibration will need to be performed after changing the capillary array or at least once a year. However, if the spectral overlap increases (i.e., excessive pull-up), then a spectral calibration will also need to be performed.

10.2.20.2.5.1.1. At the first use, thaw the 5C Matrix Mix and Matrix Dilution Buffer completely. After the first use, store the reagents refrigerated, protected from light.

10.2.20.2.5.1.2. Vortex the 5C Matrix Mix for 10–15 seconds prior to use. Add 10µl of 5C Matrix Mix to one tube of Matrix Dilution Buffer. Vortex for 10–15 seconds. Note the date of dilution on the tube.

10.2.20.2.5.1.2.1. Note: The diluted 5C Matrix Mix can be stored for up to 1 week at 2–8°C.

10.2.20.2.5.1.3. Add 10µL of the diluted 5C Matrix Mix prepared in Step 2 to 500µL of Hi-Di™ Formamide. Vortex for 10–15 seconds.

10.2.20.2.5.1.4. Load 15µL of fragment mix prepared in Step 3 into each of the wells A1-H1. After placing the septa on the plate, briefly centrifuge the plate to remove bubbles.

10.2.20.2.5.1.4.1. Note: Do not heat denature.

10.2.20.2.5.1.5. Place the plate in the 3500 series 96-well standard plate base, and cover with the plate retainer. Do not start the spectral calibration run until the oven is preheated to 60°C.

10.2.20.2.5.1.6. To perform a spectral calibration with the Promega 5-dye chemistry, a new dye set should be created. If a new dye set was created previously, proceed to step 9.

10.2.20.2.5.1.7. To create this new dye set, navigate to the Library, highlight DYE SETS and select CREATE.

10.2.20.2.5.1.8. The "Create a New Dye Set" tab will appear. Name the Dye Set, select MATRIX STANDARD for the Chemistry and select G5 TEMPLATE for the Dye Set Template. Select SAVE.

10.2.20.2.5.1.9. To perform the spectral calibration with the Promega 5-dye chemistry, go to the MAINTENANCE tab, select SPECTRAL, and under the CALIBRATION RUN tab, choose the appropriate fields: Choose MATRIX STANDARD from the Chemistry Standard drop-down menu and the new Promega 5-dye set created in step 8 (i.e., Promega G5) from the Dye Set drop-down menu. Before starting, verify that "allow borrowing" is not selected.

10.2.20.2.5.1.10. Select START RUN. If more than 1 capillary fails, the spectral calibration run will be repeated automatically up to three times. Upon completion of the spectral calibration, check the quality of the spectral in the Capillary Run Data display.

10.2.20.2.5.1.11. The Quality Value should be greater than 0.95 for each passing capillary. The Condition Value should be no greater than 13.5. Visually examine the spectral data. No or few extraneous peaks should be present as this could affect the Quality and Condition Values. After reviewing the results, choose either ACCEPT or REJECT.

10.2.20.2.5.1.12. If the spectral fails, repeat steps 1-12. If the spectral calibration still fails, consult with the DNA Technical Leader and/or a technical representative.

10.2.20.2.5.1.12.1. Note: A performance check is required after a capillary array change, spatial calibration, or spectral calibration. See 3500 Performance Checks.

10.2.20.3. Biweekly

10.2.20.3.1. Flush the pump trap with Nuclease Free water:

10.2.20.3.1.1. Fill the supplied 20 mL, all-plastic Luer lock syringe with Nuclease Free water. Expel any bubbles from the syringe.

10.2.20.3.1.2. Attach the syringe to the forward-facing Luer fitting at the top of the pump block. Hold the fitting with one hand while threading the syringe onto the fitting with the other hand.

10.2.20.3.1.3. Open the Luer fitting by grasping the body of the attached syringe and turning counterclockwise approximately one half turn to loosen.

10.2.20.3.1.4. Take approximately 30 seconds to flush 5 mL Nuclease Free water through the trap.

10.2.20.3.1.4.1. IMPORTANT! DO NOT USE EXCESSIVE FORCE when you push the syringe plunger as this may damage the trap seals.

10.2.20.3.1.5. Remove the syringe from the Luer fitting. Hold the fitting with one hand while turning the syringe counterclockwise with the other hand.

10.2.20.3.1.6. Close the Luer fitting by lightly turning clockwise until the fitting seals against the block.

10.2.20.3.1.7. Discard the flushed water and replace with new water to prevent the container from drying out.

10.2.20.4. Monthly

10.2.20.4.1. Archive data files:

10.2.20.4.1.1. Copy the .fsa files from the D: of the 3500 computer to the G: in the Run Files folder on a networked computer.

10.2.20.4.1.1.1. .fsa Files Location: This PC->(D:)->Applied Biosystems->3500->Data

10.2.20.4.1.1.2. Run Files Folder Location: (G:)->Instrument – DNA->Run Files->3500 Genetic Analyzer->Select appropriate instrument”

10.2.20.4.1.2. Ensure all files have been moved onto the G: then move the .fsa files to the recycle bin. Empty the recycle bin.

10.2.20.4.2. Defragment hard drive:

10.2.20.4.2.1. Go to START. Select AUSLOGICS DISK DEFRAG. Click Defrag.

10.2.20.4.2.2. Alternatively, go to START. Select PROGRAMS, then ACCESSORIES, then SYSTEM TOOLS, and finally DISK DEFRAGMENTER. Follow the prompts. Click Defrag.

10.2.20.5. Annually

10.2.20.5.1. A qualified representative will perform the annual maintenance. After completion of the annual maintenance, a performance check will be conducted on the instrument prior to use on casework.

10.2.20.6. 7500 Real Time PCR Instrument using HID Real-Time PCR Analysis Software v1.3 - *Monthly*

10.2.20.6.1. Perform the background calibration:

10.2.20.6.1.1. Background calibrations plates may be purchased or prepared. Prepare a background calibration plate by adding 50µL of TE buffer to each well of a 96well optical plate. Seal with optical adhesive cover and centrifuge briefly.

10.2.20.6.1.1.1. Mark the side of the plate with date and initials of the run

10.2.20.6.1.1.1.1. If used multiple times in the same day, utilize A, B, C designations to indicate number of runs on that date.

10.2.20.6.1.1.1.2. NOTE: Each plate should only be used three times.

10.2.20.6.1.2. In the 7500 software, select INSTRUMENT, then INSTRUMENT MAINTENANCE MANAGER.

10.2.20.6.1.3. In the Instrument Maintenance Manager, select the BACKGROUND tab.

10.2.20.6.1.4. In the Background tab, click START CALIBRATION.

10.2.20.6.1.5. Complete the calibration as instructed by the wizard.

10.2.20.6.1.6. Examine the data generated. Look for wells with high signal intensity (if any). These wells may need to be cleaned and the background repeated. If the same well is flagged two months in a row (i.e., if this month's calibration flags a well and the same well was flagged during the previous month's calibration), then cleaning of that well is required. If cleaning is required, clean the well(s) with a sterile swab moistened with 100% ethanol followed by a sterile swab moistened with Nuclease free water, and finished off with a dry sterile swab to remove excess liquid. Run the background calibration again.

10.2.20.6.1.7. If the signal intensity of the well(s) is still present, clean the affected well(s) with a sterile swab moistened with a 10% bleach solution. Follow with a rinse of Nuclease Free water. With a dry sterile swab, gently clean the interior of the affected wells to ensure the removal of the contaminants. Finally, using a lint-free wipe ensure any excess liquid has been cleared from the sample block. The background plate must be rerun again to determine if contamination has been removed. Record the pass/fail status of each run and the lot number of the background plate (or TE buffer) used in the calibration.

10.2.20.6.2. Check the lamp status:

10.2.20.6.2.1. In the 7500 software, select INSTRUMENT, then FUNCTION TEST, then ALL TESTS to determine the status of the instrument.

10.2.20.6.2.2. If the dialog box displays the lamp status as failed, replace the halogen lamp. See below for procedure. If the dialog box displays change soon, it is recommended to change the bulb, but not required.

10.2.20.6.2.3. If one of the other functions fails, refer to the 7500 Installation and Maintenance Guide located in the QMS Equipment List for troubleshooting steps.

10.2.20.6.3. Archive data files:

10.2.20.6.3.1. Copy the .eds files from the Experiments folder on the C: of the 7500 computer to the G: in the Run Files folder.

10.2.20.6.3.1.1. Run Files Folder Location: (G:)->Instrument – DNA->Run Files->7500 Real Time PCR->Select appropriate instrument"

10.2.20.6.3.2. Ensure all files have been moved onto the G: then move the .eds files to the recycle bin. Empty the recycle bin.

10.2.20.6.4. Defragment the hard drive:

10.2.20.6.4.1. Go to START. Select AUSLOGICS DISK DEFRAG. Follow the prompts.

10.2.20.6.4.2. Alternatively, go to START. Select PROGRAMS, then ACCESSORIES, then SYSTEM TOOLS, and finally DISK DEFRAGMENTER. Follow the prompts.

10.2.20.6.5. NOTE: A performance check is required following Background Calibration.

10.2.20.7. Semi-Annually

10.2.20.7.1. Verification of the instrument using an RNase P run will be performed semi-annually.

10.2.20.7.1.1. Alternatively, the semi-annual maintenance can be completed via temperature verification utilizing the Driftcon.

10.2.20.7.2. In this order, perform the Regions of Interest (ROI), Background Calibration (see monthly maintenance), Optical, and dye calibrations for the 4 Plexor HY dyes. Finally, an RNase P instrument verification run may be performed.

10.2.20.7.2.1. Remove the calibration plate (ROI or background) from its pouch in the Spectral Calibration Kit and thaw the plate to room temperature. Alternatively, the background plate may be prepared as stated in the monthly maintenance.

10.2.20.7.2.2. In the 7500 software, select INSTRUMENT, then INSTRUMENT MAINTENANCE MANAGER.

10.2.20.7.2.3. In the Instrument Maintenance Manager, select the tab (ROI, BACKGROUND, DYE, etc.) for the particular calibration. Click START CALIBRATION.

10.2.20.7.2.4. Complete the calibration as instructed by the wizard.

10.2.20.7.2.5. If the calibration does not pass, repeat the calibration (clean any wells as necessary). If, after a repeat, the calibration still does not pass, obtain a previously unused calibration plate and repeat the calibration. If the calibration continues to fail, consult the DNA Technical Leader and/or a technical representative.

10.2.20.7.3. To run the dye calibrators for the Plexor HY dyes:

10.2.20.7.3.1. Thaw the four Concentrated Calibrators (fluorescein, CAL Fluor® Orange 560, CAL Fluor® Red 610 and IC5) and Calibration Buffer.

10.2.20.7.3.2. Vortex the Concentrated Calibrators and Calibration Buffer to mix for 10 seconds. Pulse centrifuge each calibrator for 1-2 seconds. Do not centrifuge the spectral calibrators longer.

10.2.20.7.3.3. For each spectral calibrator, dilute each individual spectral calibrator 1:100 in Calibration Buffer, as described below.

Component	Fluorescein	CAL Fluor Orange 560	CAL Fluor Red 610	IC5
Calibrator	22µL	22µL	22µL	22µL
Calibration Buffer	2178µL	2178µL	2178µL	2178µL

10.2.20.7.3.4. Vortex the diluted spectral calibrators for 10 seconds to mix. Pulse centrifuge each calibrator for 1-2 seconds. Do not centrifuge the spectral calibrators longer.

10.2.20.7.3.5. For each diluted spectral calibrator, dispense 20µL to all 96 wells of a 96-well optical plate. Label the side of the plate skirt to designate the fluorescein, CAL Fluor Orange 560, CAL Fluor Red 610 and IC5 spectral calibration plates.

10.2.20.7.3.6. Apply a plate seal to each plate and centrifuge the plates briefly.

10.2.20.7.3.7. In the 7500 software, select INSTRUMENT, then INSTRUMENT MAINTENANCE MANAGER.

10.2.20.7.3.8. In the Instrument Maintenance Manager, select the DYE tab.

10.2.20.7.3.9. Select CUSTOM DYE CALIBRATION. Click START CALIBRATION.

10.2.20.7.3.10. Load the plate with FL calibration dye into the instrument.

10.2.20.7.3.11. Check THE CUSTOM DYE PLATE IS LOADED INTO THE INSTRUMENT.

10.2.20.7.3.12. Select START RUN.

10.2.20.7.3.13. Once the calibration run is completed, select NEXT in the bottom right of the window. The software will display the spectra. Review the spectra to confirm it is acceptable. Then, click FINISH.

10.2.20.7.3.14. If the spectra is not acceptable, repeat steps 8-13. If, after a re-run, the spectra is still not acceptable, repeat steps 1-13. If it is still not acceptable, consult with the DNA Technical Leader and/or technical representative.

10.2.20.7.3.15. Repeat for CO560, CR610 and IC5.

10.2.20.8. Annually

10.2.20.8.1. A qualified representative will perform annual maintenance. After completion of the annual maintenance, a performance check will be conducted on the instrument prior to use on casework.

10.2.20.9. As Needed

10.2.20.9.1. Replace the halogen lamp.

10.2.20.9.1.1. Power OFF, then unplug the 7500 system. Allow the instrument to cool for 15 min.

10.2.20.9.1.2. Open the access door to the 7500 system.

10.2.20.9.1.3. Remove the lamp from the instrument:

10.2.20.9.1.3.1. Slide the lamp release lever downward.

10.2.20.9.1.3.2. Firmly grasp the lamp and lift it up and out of the slotted mount.

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

10.2.20.9.1.4. Inspect the lamp for signs of failure (carbon typically coats the inside of failed lamps).

10.2.20.9.1.5. Install the new lamp into the instrument:

10.2.20.9.1.5.1. Slide the lamp release lever upward.

10.2.20.9.1.5.2. Firmly grasp the lamp, place it into the slotted mount, then carefully slide the lamp downward into place.

10.2.20.9.1.6. Close the access door.

10.2.20.9.1.7. Plug in and power ON the 7500 system.

10.2.20.9.1.8. Open the ROI Inspector dialog box:

10.2.20.9.1.8.1. In the 7500 software, select INSTRUMENT, then INSTRUMENT MAINTENANCE MANAGER.

10.2.20.9.1.8.2. In the ROI tab of the Instrument Maintenance Manager, click START MANUAL CALIBRATION.

10.2.20.9.1.9. In the ROI Inspector dialog box, select LAMP CONTROL, then IDLE.

10.2.20.9.1.10. While the instrument is running, look through grating of the access door and verify that the lamp is illuminated, then click DONE.

10.2.20.9.1.11. If the lamp is illuminated, select INSTRUMENT, then LAMP STATUS/REPLACEMENT in the 7500 software, click RESET LAMP TIMER, then click OK.

10.2.20.9.1.12. If the lamp is not illuminated, the replacement halogen lamp may be defective. Replace the lamp again. If the second lamp does not illuminate, check the instrument fuses for failure.

10.2.21. Safety

10.2.21.1. Care should be taken when handling equipment and chemicals. Personal protective equipment should be worn at all times.

10.2.21.2. Refer to SDS for chemical safety instructions and equipment user's manuals for additional safety instructions.

10.2.22. References

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10.2.22.2. 3500/3500xL Genetic Analyzer User Guide, Applied Biosystems, Foster City, CA, June 2010

10.2.22.3. 7500/7500 Fast Real-Time PCR Systems Maintenance Guide, Applied Biosystems, Foster City, CA, June 2010

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10.2.22.10. EZ1Advanced XL User Manual, Qiagen, Hilden, Germany, May 2009

10.2.22.11. EZ2 Connect and EZ2 Connect Fx User Manual, Qiagen, Hilden, Germany, March 2024

10.2.22.12. EZ2 Connect Fx Recovery Procedure Instruction Manual, Qiagen, Hilden, Germany, June 2023

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10.2.22.16. NewClassic Balances MS-S / MS-L Models. Operating Instructions. Mettler-Toledo AG 01/2011.

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10.2.22.18. Plexor HY System for the Applied Biosystems 7500 and 7500 FAST Real-Time PCR Systems Technical Manual, Promega, Madison, WI, May 2013

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10.2.22.20. PowerPlex 5C Matrix Standard, Promega, Madison, WI, October 2015

10.2.22.21. Purifier® Logic® Series Biological Safety Cabinets User's Manual. Labconco Corporation 2010.

10.2.22.22. Purifier® Non-Ventilated PCR & Tissue Culture Enclosures User's Manual. Labconco Corporation 2007.

10.2.22.23. QIAcube User Manual, Qiagen, Hilden, Germany, June 2008

10.2.22.24. QIAGEN QIAgility® User's Manual. June 2013.

10.2.22.25. Thermomixer comfort / Thermomixer R Operating Manual. Eppendorf 2007.

10.2.22.26. Veriti Thermal Cyclers User Manual, Applied Biosystems, Foster City, CA, June 2010

10.3. Performance Checks

10.3.1. Performance checks of critical instruments and equipment will be conducted per specifications outlined in this section of the Forensic Biology Quality Manual.

10.3.2. As Needed Performance Checks

10.3.2.1. Performance checks of critical instruments or equipment will also be performed when at least one of the following occurs:

10.3.2.1.1. when an additional validated instrument is added (8.6.1)

10.3.2.1.2. when modifications to an instrument are made that do not affect the analytical portion of the instrument (8.6.2) (i.e., new capillary array)

10.3.2.1.3. following software upgrades that do not impact interpretation, the analytical process, or sizing algorithms (8.7.2)

10.3.2.1.4. upon the return of the instrument/equipment when loaned outside of the laboratory

10.3.2.1.5. upon request by the DNA Technical Leader

10.3.3. Annual Performance Checks

10.3.3.1. A performance check will be conducted annually on the following critical instruments and equipment:

10.3.3.1.1. Eppendorf Pipette, Model Research Plus 0.1-2.5 (by a Qualified Technician)

10.3.3.1.2. Eppendorf Pipette, Model Research Plus 0.5-10 (by a Qualified Technician)

10.3.3.1.3. Eppendorf Pipette, Model Research Plus 2-20 (by a Qualified Technician)

10.3.3.1.4. Eppendorf Pipette, Model Research Plus 10-100 (by a Qualified Technician)

10.3.3.1.5. Eppendorf Pipette, Model Research Plus 20-200 (by a Qualified Technician)

10.3.3.1.6. Eppendorf Pipette, Model Research Plus 100-1000 (by a Qualified Technician)

10.3.3.1.7. Eppendorf Pipette, Model Research Plus 8-Channel 0.5-10 (by a Qualified Technician)

10.3.3.1.8. Eppendorf Pipette, Model Repeater Stream (by a Qualified Technician)

10.3.3.1.9. Extraction Robot, Model EZ1 Advanced XL

10.3.3.1.10. Extraction Robot, Model EZ2 Connect Fx

10.3.3.1.11. DNA Purification Robot, Model QIAcube

10.3.3.1.12. DNA Transfer and Normalization Robot, Model QIAgility

10.3.3.1.13. Thermal Cycler, Model Veriti

10.3.3.1.14. Real Time PCR System, Model 7500

10.3.3.1.15. Genetic Analyzer, Model 3500

10.3.3.1.16. Driftcon (by a Qualified Technician)

10.3.3.1.17. Thermomixers

10.3.4. Repair, Service and/or Calibration

10.3.4.1. A performance check will also be performed following repair, service or calibration of the following equipment prior to using the equipment on casework analysis:

10.3.4.1.1. Extraction Robot, Model EZ1 Advanced XL and EZ2 Connect Fx

10.3.4.1.2. DNA Purification Robot, Model QIAcube

10.3.4.1.3. DNA Transfer and Normalization Robot, Model QIAgility

10.3.4.1.4. Thermal Cycler, Model Veriti

10.3.4.1.5. Real Time PCR System, Model 7500

10.3.4.1.6. Genetic Analyzer, Model 3500

10.3.4.2. When the transport of measuring equipment and/or critical instruments used by the MNPD-CL FB unit is necessary with the intention of the equipment being returned, precautions will be taken. When shipping the item(s), recommendations from the manufacturer or vendor receiving the equipment will be followed. If no recommendations are received, care will be taken in packing the equipment to include packaging material to stabilize/protect the equipment in transport (i.e., bubble wrap, Styrofoam, etc.). Upon the return of the equipment, the item will be decontaminated prior to returning the equipment to the lab in which it will be operated. A performance check will be conducted to ensure the operation of the equipment prior to its use on casework.

10.3.5. Instructions for Performance Checks

Instrument	Performance check instructions	Requirements of passing results
3500 using Data Collection Software	Set up a 96-well plate consisting of one column of ladders (1), one column of previously run positive controls (2), and one column of previously run negative controls (3); totaling 3 columns. See the Capillary Electrophoresis section of the MNPD-CL Forensic Biology TPM for the procedure.	Controls and samples must result in concordant typed profiles. Column of Negative controls must pass per specifications outlined in the Data Analysis section of the Forensic Biology Technical Procedures Manual.

Instrument	Performance check instructions	Requirements of passing results
7500 using HID RT PCR Software	Option 1: A 96 well plate set up with 2 columns of Standards (Standards 1-7 in wells A through G, respectively) analyzed within the Plexor software. See MNPD- CL Forensic Biology TPM section Quantitation for protocol. Option 2: An RNAse P Instrument Verification Plate may be run as a performance check.	Option 1: The Slope and R2 values of the standard curve must fall within ranges indicated within the Forensic Biology Technical Procedure Manual. The Y-intercept (b) for both the autosomal and Y standard curve must fall within the range noted in the MNPD Plexor Validation (Auto: 22.26 to 24.72; Y: 23.55 to 25.40). Option 2: The instrument passes the verification if the analyzed data demonstrates that the instrument distinguishes between 5,000 and 10,000 genome equivalents with a 99.7% confidence level.
Qiagen EZ1/2 and QIAcube	Conducted using, at minimum, one known DNA sample and one reagent blank. QIAcube instruments must be performance checked using a differential extraction, while EZ1/2 instruments can be performance checked through either differential or non-differential extraction methods. See the Non-Differential Extraction Procedures or Differential Extraction Procedures section of the MNPD-CL Forensic Biology TPM for the protocol.	The quantitation result must be consistent with expected results. The DNA sample must possess an autosomal concentration ≥ 10pg/ul and the reagent blank must be negative.
QIAgility using Operating Software v4.17.1	Conducted using, at minimum a known DNA sample and a reagent blank. The applicable protocols for which the instrument is used will be executed (i.e., Quantitation Set-up and Amplification Set-up/Normalization, GenTegra	Ensure the QIAgility performs the liquid handling steps appropriately. All typed profiles must be consistent with expected results and a clean reagent blank must be achieved

Instrument	Performance check instructions	Requirements of passing results
	Sample Preservation, or Capillary Electrophoresis set-up).	per specifications of the Data Analysis section of the Forensic Biology Technical Procedures Manual.
Thermometers (in fridges, freezers, and thermal shakers used to store or process DNA samples)	Once a year using a NIST certified thermometer applicable to their respective temperature ranges. NIST Traceable Thermometers will be calibrated or purchased on an annual basis. Certification records will be stored in the QMS.	A thermometer will be considered satisfactory if it falls within +/- 1 degree Celsius of the certified NIST Traceable thermometer.
Thermal Cycler	Use a temperature verification system, either by an outside vendor or internally. If conducting the temperature verification internally, see the instructions outlined in Appendix E of this manual.	Ensure the measured step, accuracy and uniformity results for each well are within the software specifications or passing range.

10.3.5.1. Performance checks are documented within the QMS. Documentation of the approval/rejection is performed by a qualified analyst or the DNA Technical Leader. If a performance check fails to produce passing results, the instrument/equipment will be tagged out and documented in an error log. The failure will be investigated to determine any troubleshooting measures (e.g., operation manuals, contact technical support, network with the community, etc.) and rerun. If the performance check continues to fail after troubleshooting, a vendor will be contacted for a quote regarding service, replacement, and/or recalibration.

10.4. Records Retention

10.4.1. Records of service, maintenance, and/or calibrations; performance checks; and thermometer certifications are maintained in the QMS per the policy for Quality Record retention outlined in the MNPD-CL Quality Manual.

11. Case Documentation

11.1. A Forensic Biology case record will be generated and maintained for each case received into the MNPD-CL Forensic Biology unit. For any evidence received within the Forensic Biology Unit, a case record must be generated. The case record must contain a chain of custody, the Request for Service and a Forensic Biology Report. Other documentation will depend upon the extent and type of analysis conducted. An MNPD-CL Forensic Biology case record may include various components that are located in the MNPD-CL LIMS and/or the secure MNPD-CL network drives.

11.2. Technical Records

11.2.1. Some documentation of the processing of the evidence within the Forensic Biology Unit is provided in the form of an electronic case file folder. Components of the electronic case file folder may include, but are not limited to:

11.2.1.1. Case file PDF

11.2.1.2. Review Form(s)

11.2.1.3. Photos

11.2.1.4. CODIS Information

11.2.2. The release of an electronic case file will adhere to the policy set forth in 11.3 of this document and the MNPD-CL QM.

11.2.3. Examination documentation includes any documentation used to reach a conclusion. The examination documentation must contain sufficient detail to allow an examiner, other than the one reporting the conclusion, to evaluate and interpret the data that supports the conclusion. Examination documentation may include, but is not limited to:

11.2.3.1. Tests conducted and results

11.2.3.2. Documentation of the standards and controls used

11.2.3.3. Digital images

11.2.3.4. Data and calculations

11.2.3.5. Notes

11.2.4. Examination documentation shall include the identity of personnel involved in processing the evidence and samples and witnessing steps.

11.2.5. Notes may be presented in standard examination forms, photographs and/or the LIMS data extension fields. Standard forms ensure the documentation of significant information within the case file. The forms also provide a consistent format among the

examiners/technicians which aid in the review process. Forms used to provide documentation are:

11.2.5.1. Serological Exam Worksheet

11.2.5.2. Evidence Inventory Worksheets

11.2.5.3. Consolidated Screening Form

11.2.5.4. General Case Notes Form

11.2.5.5. Batch Workbook(s)

11.2.5.6. Statistical Worksheet

11.2.6. Notes will document (i.e., written description, photograph) the container, items, other packaging, type of seal, and any damage to the item and/or seal.

11.2.7. The approximate location of areas tested should be documented.

11.2.8. Obvious staining should be noted. This may be in the form of a photograph.

11.2.9. Document whether or not a stain/sample is consumed.

11.2.10. Recommended documentations, where applicable, include, but are not limited to:

11.2.10.1. Color

11.2.10.2. Size

11.2.10.3. Brand

11.2.10.4. Designs

11.2.10.5. Fabric separations

11.2.10.6. Pocket contents

11.2.10.7. Any change made to the evidence

11.2.11. Lot numbers of the reagents used will be documented in the examination documentation.

11.2.12. Justification for any “inconclusive” results will be documented in the examination documentation.

11.2.13. Digital images utilized for examination documentation will be marked with the case number, item number(s), date, and examiners initials. This may be accomplished by capturing the information in the image or marking the image after the image is taken.

11.2.14. “Shorthand” may be used for documentation. Acceptable abbreviations are listed in Appendix C Abbreviations.

11.2.15. See the definition for “Records (administrative records)” in the MNPD-CL QM.

11.3. Reporting Elements

11.3.1. MNPD-CL Forensic Biology Reports include the following elements:

11.3.1.1. Date issued

11.3.1.2. Case Identifier

11.3.1.3. Description of the evidence examined

11.3.1.4. Description of the DNA technology used

11.3.1.5. Locus or amplification system (Serology Reports exempt)

11.3.1.6. Results and/or conclusions

11.3.1.7. Quantitative or qualitative interpretative statement

11.3.1.8. Disposition of evidence

11.3.1.9. Signature and title of person accepting responsibility for the content of the report

11.4. Confidentiality

11.4.1. The MNPD-CL Forensic Biology Unit adheres to policies and procedures in order to maintain the confidentiality of reports, case files, DNA records, and databases.

11.4.2. Confidentiality and security policies and procedures regarding the case file will follow the practices outlined in MNPD-CL QM and Guiding Principles of Professional Responsibility for Forensic Service Providers and Forensic Personnel. All requests for information within the case file must be in writing. The request must be specific as to the information desired. A copy of each response must be retained as part of the case record.

11.4.3. Confidentiality and security policies and procedures regarding reports will follow the practices outlined in MNPD-CL QM.

11.4.4. Confidentiality and security policies and procedures regarding the DNA database are outlined in the MNPD-CL CODIS Manual.

12. Review

12.1. Documentation

12.1.1. Technical and administrative reviews will be conducted on 100% of the case files and reports issued by the MNPD-CL Forensic Biology Unit and will follow the procedures detailed in the MNPD-CL QM.

12.1.2. The technical and administrative review will be documented on the review forms and/or in the LIMS.

12.1.3. The required qualifications of the technical reviewer are outlined in Section 5.5 Technical Reviewer qualifications of this manual. The qualifications of each staff member conducting technical reviews may be found in their respective Personnel Files.

12.2. Technical Review Elements

12.2.1. The elements of the technical review and administrative review are outlined in various review forms used by the MNPD-CL FB unit.

12.3. Administrative Review Elements

12.3.1. The elements of the technical review and administrative review are outlined in various review forms used by the MNPD-CL FB unit.

12.4. Discrepancy Resolution

12.4.1. The procedure for addressing unresolved discrepant conclusions between analysts and reviewer(s) is outlined in the MNPD-CL QM.

12.5. Database Matches

12.5.1. The procedure for verification and resolution of database matches is outlined in the CODIS Manual.

13. Proficiency Testing

13.1. Personnel

13.1.1. The MNPD-CL has a proficiency testing program with general oversight by the Quality Manager (see the MNPD-CL QM). The Forensic Biology Unit adheres to the MNPD-CL proficiency testing program and the following:

13.1.2. MNPD-CL Forensic Biology staff members performing technical procedures or reviews on DNA casework will undergo a semi-annual external proficiency test in each technology performed to the full extent which they participate in DNA casework. The external proficiency testing will be an open proficiency testing program and will be submitted to the proficiency testing provider in order to be included in the provider's published external summary report. An analyst's ability to assess CODIS Eligibility will be annually tested through the "Annual Review of DNA Accepted at NDIS" exam administered by the FBI.

13.1.3. Analysts are proficiency tested in each technology, for which they are qualified, at least once per calendar year, attempting all CODIS core loci or CODIS core sequence ranges once per calendar year.

13.1.4. Analysts are proficiency tested on each typing test kit, for which they are qualified, at least once per calendar year.

13.1.5. Individuals are proficiency on at least one method in each methodology at least once per calendar year.

13.1.6. The MNPD-CL FBU can utilize technicians and/or a team approach to process routine casework and reserve the right to use the same approach on proficiency tests. However, each analyst authorized in interpretation and reporting is assigned a proficiency test to complete the interpretation and reporting aspects.

13.1.6.1. Communication:

13.1.6.1.1. Scientists authorized to only perform DNA extraction, quantitation, normalization, amplification, CE, and/or data analysis with the GMID-X software are termed DNA processors within the Unit.

13.1.6.1.1.1. In casework the reporting analyst and batch reviewer are different individuals. The reporting analyst is involved in the batch review process to ensure they agree with the work performed by the DNA processors (e.g., allele edits). During this step of batch review, or prior to, if a sample needs to be reworked (e.g., re-amplification) the DNA processor has a conversation with the reporting analyst to ensure agreement on a course of action. These types of conversations will be maintained during proficiency testing.

13.1.6.1.2. The DNA results produced during a proficiency test by a DNA processor are not reported in LIMS. The process ends with batch review. As such, the assigned batch reviewer will act as both the reporting analyst (sans report) and batch reviewer during a proficiency test. If the DNA processor is authorized through data analysis with GMID-X they will analyze the data in accordance with their authorizations (i.e., perform data analysis). If the DNA processor is not authorized in data analysis with GMID-X (i.e., authorized in extraction through to CE only), the batch reviewer will perform the data analysis to include making any necessary allele edits. On the “Processing Batch Form and Review" document (i.e., batch review form) the DNA processor will complete the Forensic Scientist column for processes they are authorized to perform.

13.1.6.2. General Review Process:

13.1.6.2.1. Analysts performing the technical and administrative reviews must review proficiency casework in the same manner as normal casework. Questions that result from the review will be documented as they would for casework. For proficiency tests, the reviewer only has the responsibility to question, not to enforce policy compliance. Policy compliance is ultimately the responsibility of the reporting analyst.

13.1.6.2.1.1. For example: After completing their review of the proficiency case, the reviewer questions (documented in pdf case file comments) why the reporting analyst did not perform XYZ task as required by ABC policy. The reviewer's responsibility is now complete. The reporting analyst is responsible for either justifying their actions or amending their actions.

13.1.7. Individuals whose sole responsibility is technical review are proficiency tested in the technical review of each technology and typing test kit for which they are qualified to perform technical review, covering the CODIS core loci or CODIS core sequence ranges attempted, at least once per calendar year.

13.1.8. Analysts, newly qualified in DNA casework, will enter the external proficiency test program within eight months of the date of their qualification.

13.2. Proficiency Test Provider

13.2.1. The external proficiency test provider utilized by the MNPD-CL Forensic Biology Unit is documented in the QMS. The proficiency test program, to include the qualification of the provider and accessibility of the test results, are described in the MNPD-CL QM.

13.3. Tracking

13.3.1. The due date of the external proficiency test will be used for tracking compliance with the semi-annual proficiency testing requirement. Proficiency Tests are tracked in the QMS.

13.4. Records Retention

13.4.1. A proficiency test file (either paper or electronic) will be generated. At minimum, the following will be included in the file:

13.4.1.1. Test set identifier

13.4.1.2. Identity of analyst and other participants, if applicable

13.4.1.3. Date of analysis and completion

13.4.1.4. Copies of all data and notes supporting the conclusions

13.4.1.5. Proficiency test results

13.4.1.6. Any discrepancies noted

13.4.1.7. Corrective actions taken

13.5. Evaluation

13.5.1. Proficiency tests are evaluated by the DNA Technical Leader using the QMS.

13.6. Results

13.6.1. Proficiency test participants are informed of their final test results. Documentation of this notification is included in the QMS.

13.6.2. If the Casework CODIS Administrator is designated as someone other than the DNA Technical Leader (see Forensic Biology Organizational Chart), the Casework CODIS Administrator will be notified of all non-administrative discrepancies that affect typing results and/or conclusions at the time of discovery. This notification will be documented in the proficiency test file.

14. Corrective Action

14.1. The policies and procedures regarding corrective actions are outlined in the MNPD-CL QM. Corrective action plans for the Forensic Biology Unit will not be implemented without the approval of the DNA Technical Leader. This approval is documented in the MNPD-CL compliance software. The casework CODIS administrator will be notified when the nonconformity impacts DNA records entered into CODIS.

15. Audits – The MNPD-CL Forensic Biology Unit participates in MNPD-CL (laboratory wide) internal and external audit(s). (See the MNPD-CL QM and ANAB ISO/IEC 17025 Forensic Science Testing Laboratories Accreditation Requirements). In addition, the Forensic Biology unit adheres to the following:

15.1. Schedule

15.1.1. The MNPD-CL Forensic Biology Unit will be audited annually in accordance with the most current Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories.

15.2. External Audits

15.2.1. At least once every two years, an external QAS audit will be conducted. The audit team will be comprised of auditor(s) outside of the MNPD-CL and will have at least one

team member who is or has been an analyst previously qualified in the MNPD-CL Forensic Biology Unit's current DNA technologies and platforms. The qualifications of the auditor will be maintained with audit documentation (see 15.6).

15.2.2. External NDIS audits are performed periodically by the FBI.

15.3. Internal Audits

15.3.1. Internal audits may be performed on years external audits are not performed or at the request of the FB Supervisor, DNA Technical Leader, MNPD-CL Quality Manager, MNPD-CL Director, or MNPD Administration. The audit team will be comprised of qualified auditor(s) from within the MNPD-CL. At least one team member will be or will have been an analyst previously qualified in the MNPD-CL Forensic Biology Unit's current DNA technologies and platform. The qualifications of the auditor will be maintained with audit documentation (see 15.6).

15.4. Audit Document

15.4.1. QAS audits will be conducted utilizing the current FBI DNA Quality Assurance Standards Audit Document.

15.5. Audit Review

15.5.1. All audit documents and, if applicable, corrective action(s) will be reviewed by the DNA Technical Leader to ensure that findings, if any, were appropriately addressed. This review will be documented in the QMS. Internal and external documentation, and if applicable, corrective action(s) will be provided to the casework CODIS administrator.

15.6. Records Retention

15.6.1. Audit documentation will be maintained electronically within the QMS. Retention of the audit files is addressed in section 3.2 Document Retention Policies of this manual.

16. Professional Development

16.1. The qualifications of the Forensic Biology staff are maintained by attending in-person or virtual seminars, courses, professional meetings and training, in addition to reviewing scientific literature.

16.2. Continuing Education

16.2.1. Forensic Biology Scientists and Technicians authorized in DNA casework, the DNA Technical Leader, and the Casework CODIS Administrator will maintain technical qualifications through annual continuing education. Eight cumulative hours of continuing education relevant to developments of DNA typing may be obtained

internally, externally, or through multimedia or Internet routes. Documentation of the continuing education is maintained in the QMS. Specific requests for continuing education may be made by submitting a MNPD Form 445 to the Supervisor and/or a DNA Training Approval workflow in QMS to the DNA Technical Leader. Activities identified under QAS that do NOT constitute continuing education are:

16.2.1.1. Activities that are required for establishing an individual's competency

16.2.1.2. Reading scientific literature and subsequent discussions (e.g., journal club, article presentation)

16.2.2. Internal Continuing Education

16.2.2.1. Internal training is training conducted by a member of the MNPD-CL. The presenter of the internal training will file a DNA Training Approval workflow in the QMS. The presenter's CV will be included in the workflow. Attendees will complete a Continuing Education workflow, uploading an attendance list along with any certificates in the QMS.

16.2.3. External Continuing Education

16.2.3.1. External continuing education is documented through the Continuing Education workflow. Attendees will maintain a copy of at least one of the following: certificate or documentation indicating completion of the training, program agenda/syllabus, or travel documentation in the QMS.

16.2.4. Multimedia or Internet Continuing Education

16.2.4.1. Approval of the content of any multimedia or internet training will be documented through the DNA Training Approval workflow in the QMS. Attendees will document completion of the training through a Continuing Education workflow.

16.2.5. Scientific Literature

16.2.5.1. Forensic Biology staff members authorized to perform casework will be responsible for periodically reviewing scientific literature applicable to DNA analysis. Individuals will review at least four pieces of literature per year. Readings are documented in the QMS and/or training checklists. Individuals may volunteer or be asked to present articles at unit staff meetings. The MNPD-CL Forensic Biology staff has access to both electronic and physical books and/or journal articles.

16.3. Testimony Review

16.3.1. Forensic Biology Staff will be responsible for keeping a log of court appearances in the QMS or on the server. Each person testifying in DNA analysis is required to be monitored annually. Testimony monitoring documentation will be maintained in the QMS. The procedure for Court Testimony reviews is outlined in the MNPD-CL QM. Analysts who do not testify during the calendar year will be marked as such in the QMS.

17. Outsourcing Ownership

17.1. In the event that MNPD uses a vendor laboratory to provide DNA services which will provide data that will be entered into MNPD-CL Forensic Biology Unit's DNA Database, the standards below will be followed.

17.2. Vendor Laboratory

17.2.1. The outsourcing laboratory must hold current international forensic accreditation through ANAB. Documentation of the accreditation and compliance with FBI QAS for DNA Testing Laboratories will be maintained by the MNPD-CL Forensic Biology Unit. The vendor laboratory's external audit documentation, along with responses and/or corrective actions for any findings will be reviewed by the MNPD-CL DNA Technical Leader (TL) and maintained in the QMS, unless the FBI's coordinated visit is being utilized as the on-site visit.

17.3. Technical Specification Approval

17.3.1. The DNA Technical Leader will document approval of the technical specifications of the outsourcing agreement with a vendor laboratory before it is awarded. The date of approval is documented in the QMS.

17.3.2. No DNA data will be uploaded to the MNPD-CL Forensic Biology Unit's DNA database prior to the documented approval of the technical specifications of the outsourcing agreement and/or documented approval of acceptance of ownership of the DNA data by the DNA Technical Leader.

17.3.3. Ownership with No Outsourcing agreement

17.3.3.1. Although highly discouraged, MNPD-CL FBU may take ownership of data when no outsourcing agreement exists between either the law enforcement agency, the vendor laboratory or MNPD-CL. In order for this to take place, the MNPD-CL DNA TL must document the following prior to taking ownership:

17.3.3.1.1. Approval of the MNPD-CL FBU casework CODIS Administrator and written permission from the NDIS Custodian

17.3.3.1.2. Approval of the technical specifications of testing

17.3.3.1.3. Review of the documentation of or conduct an on-site visit within 18 months of the conducted analysis in accordance with 17.4.2 (listed in FBI QAS 2025).

17.4. Integrity of DNA Data

17.4.1. A review will be performed on the data received from the outsourcing laboratory prior to upload to the state DNA database.

17.4.2. Outsourced DNA data may be searched in the database prior to the completion of an ownership review. At minimum, verification of specimen eligibility and correct specimen category must be completed prior to entry into the DNA database. This verification may be performed by an MNPD-CL FBU analyst, the CODIS Administrator, the Alternate CODIS Administrator, or an MNPD-CL FBU technical reviewer. This verification will be documented on a review form and/or in the LIMS.

17.4.3. The data review of the outsourcing laboratory's data will be performed by an MNPD-CL FBU analyst or technical reviewer who is currently qualified in the technology, platform, and typing test kit used to generate the data. The data review includes, at minimum, a review of the following elements:

17.4.3.1. All DNA types to verify that the profiles are supported by the raw and/or the analyzed data (e-grams or images)

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

17.4.3.3. Final report to verify results/conclusions are supported by the data and that each tested item (or its probative fractions) are reported

17.4.3.4. Verification of the DNA types, eligibility, and correct specimen category by a current CODIS user prior to being entered in CODIS

17.4.4. The review will be documented on a review form and/or in the LIMS.

17.4.5. MNPD-CL FBU does not accept data generated by Rapid DNA from vendor laboratories.

17.5. On-site Visit

17.5.1. Initial and annual on-site visits to the vendor laboratory will be conducted.

17.5.2. The initial on-site visit will be performed prior to the vendor laboratory performing any casework analysis for the MNPD-CL. The initial visit may be conducted by either the DNA Technical Leader, or a designated employee of the MNPD-CL FBU, who is

qualified or previously qualified in the technology, platform and typing amplification test kit that will be used by the vendor laboratory to generate data for the MNPD-CL. Alternatively, the MNPD-CL may accept the on-site visit coordinated by the FBI.

17.5.3. When the outsourcing agreement extends beyond a year, an annual on-site visit will occur each calendar year, at least 6 months and no more than 18 months apart. The annual site visit may be conducted by the DNA Technical Leader or a designated employee of the MNPD-CL FBU, who is qualified or previously qualified in the technology, platform and typing amplification test kit that will be used by the vendor laboratory to generate data for the MNPD-CL. Alternatively, the MNPD-CL TL may accept an on-site visit coordinated by the FBI or conducted by another NDIS participating laboratory using the same technology, platform and typing amplification test kit that will be used by the vendor laboratory to generate data for which the MNPD-CL FBU will take ownership.

17.5.4. Documentation of on-site visits are maintained in the QMS.

18. Rapid DNA

18.1. The MNPD-CL does not have a Forensic Rapid DNA Program.

18.1.1. The MNPD-CL does not have Forensic Rapid DNA Program with a Rapid DNA partner agency.

18.2. The MNPD-CL does not conduct Rapid DNA Analysis.

18.3. The MNPD-CL does not conduct Modified Rapid DNA Analysis.

19. References

19.1. ANAB ISO/IEC 17025:2017. Forensic Science Testing Laboratories: Accreditation Requirements

19.2. Benschop, C.C.G., et al. (2010). Post-coital vaginal sampling with nylon flocked swabs improve DNA typing. Forensic Biology Science International: Genetics, 4, 115-121.

19.3. Butler, J.M. (2012) Advanced Topics in Forensic Biology DNA Typing: Methodology. San Diego:Elsevier Academic Press.

19.4. DNA Advisory Board (1998). Quality assurance standards for Forensic Biology DNA testing laboratories. Forensic Biology Science Communications, (2)3

19.5. ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories.

19.6. Lee, H.C. & Ladd, C. (2001). Preservation and Collection of Biological Evidence. Croatian Medical Journal, 42, 225-228.

19.7. Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories (2020)

19.8. The Guidance Document for the FBI QAS for Forensic DNA Testing and DNA Databasing Laboratories (2020).

19.9. Sweet, D., et al. (1997). An improved method to recover saliva from human skin: The double swab technique. Journal of Forensic Biology Sciences, 42, 320-322.

19.10. United States Department of Labor (lack of) universal precautions

Appendix A Evidence Handling

1. General Safety

1.1. Due to the nature of the evidence received within the Forensic Biology Unit, employees will routinely encounter potential biological hazards. In addition to biological hazards, an employee within the unit will also encounter various chemical and physical hazards. Below are general safety requirements to be followed to ensure the safety of laboratory personnel. In addition to these general rules, more specific practices are covered, as applicable, in the MNPD-CL Forensic Biology Technical Procedures and the MNPD-CL Safety Plan.

1.1.1. Employees should familiarize themselves with any potential physical, biological, and/or chemical hazards prior to performing job responsibilities.

1.1.2. Laboratory personnel will be aware of the location of the nearest eyewash station and safety shower when handling hazardous materials.

1.1.3. Follow the safety recommendations provided by the manufacturer when handling equipment, chemicals, and reagents.

1.1.3.1. SDS information can be found in the QMS.

1.1.3.2. Additional information may be located in the DNA Manuals folder - L:\Crime Lab Network Drive\Forensic Biology\Forensic Biology Unit\Additional information\DNA Manuals.

1.1.4. Chemicals and reagents will be disposed of as dictated in the SDS.

1.1.5. All potential biological evidence should be handled using universal precautions (http://www.osha.gov/SLTC/etools/hospital/hazards/univprec/univ.html and MNPD-CL Safety Plan).

1.1.5.1. The minimum required personal protective equipment when working in the laboratory is a lab coat and gloves.

1.1.5.2. When pouring liquids, eyes should be protected.

1.1.5.3. Wash hands after removing gloves.

1.1.5.4. Wash hands immediately following possible exposure to blood or other possible infectious material.

1.1.5.5. Do not recap needles or scalpels.

1.1.5.6. Decontaminate areas immediately following procedures.

1.1.5.7. Dispose of all glass and/or sharps in sharps containers.

1.1.5.8. Materials containing biological waste must be disposed of in waste containers designated for biohazards.

1.1.5.9. Eating, drinking, or applying cosmetics is prohibited in laboratory working areas.

1.1.5.10. The DNA office areas are considered clean areas. Evidence, chemicals, and equipment are prohibited in these areas.

2. Creating Sub-items

2.1. In the Forensic Biology Unit, sub-itemization may be required. The process for creating sub-items in association with a parent item is described in the MNPD-CL ERU SOP and Help Menu in the LIMS. The sub-item inherits the chain of custody (COC) of the parent item. Once established as a sub-item, the item will adopt its own COC.

2.1.1. When multiple items are submitted under a single evidence number, the items may be sub-itemized.

2.1.2. When preservation swabs are collected from items of evidence, a sub-item will be created.

2.1.3. When evidence is collected and repackaged separately, a sub-item will be created.

2.1.4. Sub-items will be listed on the Forensic Biology Report.

3. Sample Selection

3.1. Sample selection for serological screening and DNA analysis will be based upon information provided to the examiner and the examiner's training and experience used to evaluate the condition of the sample and/or preliminary testing results of the sample. The decision trees outlined in the following sections should be routinely used in processing certain samples; however, circumstances may warrant slight variations from routine processing.

3.1.1. Control swabs submitted with the evidence and areas adjacent to the stain will not be routinely tested.

3.1.2. When appropriate, stains may be tested for the presence of multiple body fluids.

3.1.3. Follow the guidelines outlined in Section 11 of this manual for information documented in notes.

3.1.4. Note if stain or item is unsuitable for PCR DNA testing.

3.1.5. As appropriate, conduct PCR DNA testing on the stain.

3.2. If review of the case information results in uncertainty of what test methods should be utilized, the analyst will make attempts to resolve the ambiguity by contacting the customer. If the ambiguity cannot be resolved, the analyst will discuss the situation with their

Supervisor. Ultimately, if the ambiguity cannot be resolved, the case request will be cancelled.

3.2.1. If the case in question is a proficiency test, the analyst cannot request assistance from the vendor and/or Supervisor, therefore the ambiguity cannot be resolved. Additionally, the proficiency test cannot be cancelled due to the case scenario's ambiguity. As a result, the proficiency test's evidence items will move forward to both blood and semen screening.

4. Blood

4.1. Blood samples may be submitted to the lab as known standards or as evidentiary items. Blood samples submitted as reference standards may be received as liquid or dried stains. Suspected blood submitted on evidentiary items is routinely a dried sample.

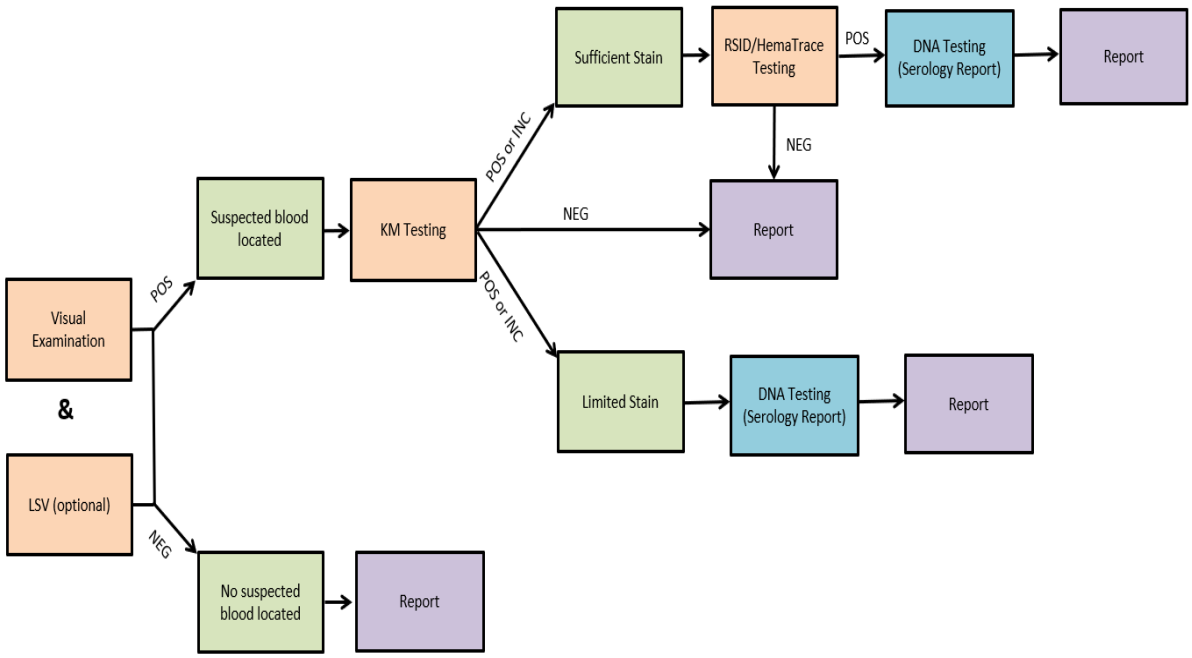
4.2. Liquid Samples

4.2.1. If liquid blood is submitted as a reference standard, a portion of the sample will be dried for use in testing following the procedure located in MNPD-CL FB TPM Sample Preparation.

4.3. Evidentiary Bloodstains

4.3.1. When screening evidentiary items for the possible presence of blood, the examiner should follow the Decision Tree for Blood Screening on Bulk Evidence.

4.3.2. Decision Tree for Blood Screening on Bulk Evidence



4.3.2.1. When swabs are submitted for blood screening, note visual examination and proceed to KM testing in decision tree.

4.3.2.2. Multiple blood stains are numerically identified as 1, 2, 3, etc.

4.3.2.3. Multiple stains may be treated as separate stains or the same stain if deemed to be part of a pattern.

4.3.2.4. When multiple stains test KM positive, analysts can choose to further test all stains or just the stains that they would take forward to DNA testing if there is a positive HemaTrace result. If the stains the analysts choose test negative, then analysts would continue to test other stains until there is a positive result, or they all test negative.

4.4.Testing

4.4.1.Any serological testing will adhere to the appropriate protocol(s) outlined in the MNPD-CL Forensic Biology Technical Procedures Manual.

5. Suspected Semen

5.1. The process for screening evidentiary items for the possible presence of semen is dependent upon the type of evidence examined. These processes are applicable to items in which semen is the body fluid of interest.

5.2.Liquid Samples (includes oral rinses and condoms containing liquid)

5.2.1. If liquid samples are submitted for examination for the presence of semen, a portion of the sample will be collected and dried for use in testing or centrifuged and examined for spermatozoa following the procedure located in MNPD-CL FB TPM Sample Preparation.

5.3. Dried Stains

5.3.1. Swabs, Sexual Assault Evidence Collection Kits (SAECK's), and Underpants

5.3.1.1. Unless circumstances of a case warrant otherwise, the Forensic Biology Unit requests that swabs and/or SAECK's be the initial submission in sexual assault investigations.

5.3.1.2. For cases reported to the lab as a single suspect case, in which the alleged victim reports no recent consensual sex, the analyst may limit the number of items initially tested.

5.3.1.3. When a piece of evidence is represented by only one Copan swab, testing will begin with Micro Slide Prep and proceed to Differential Extraction.

5.3.1.4. For Screening Swabs, SAECK's, and Underpants (Female Victim/Male Suspect)

5.3.1.4.1. When screening swabs, SAECK's, and/or underpants in investigations involving female victims and male suspects, DNA quantitation will be routinely used to screen for the presence of male DNA. This section, Screening Swabs, SAECK's, and Underpants (Female Victim/Male Suspect), should be routinely used to process these sample types; however, on occasion, circumstances may render deviations from this practice.

5.3.1.4.2. If swabs are submitted in the SAECK, no serology screening is necessary. A cutting from each cotton swab, or a nylon-flocked swab, from an item of evidence will be taken directly to Differential Extraction.

5.3.1.4.3. If only one cotton swab is collected for a piece of evidence, half of the swab will be cut for Differential Extraction and the other half preserved for future testing.

5.3.1.4.4. If only one Copan swab is submitted for a piece of evidence, the swab will be used for Micro Slide Prep and potential DNA testing (i.e., only one vaginal swab submitted – a Micro Slide Prep will be created before the swab is sampled and consumed for DNA).

5.3.1.4.5. Underpants should be AP mapped.

5.3.1.4.5.1. The entire inside of underwear should be tested.

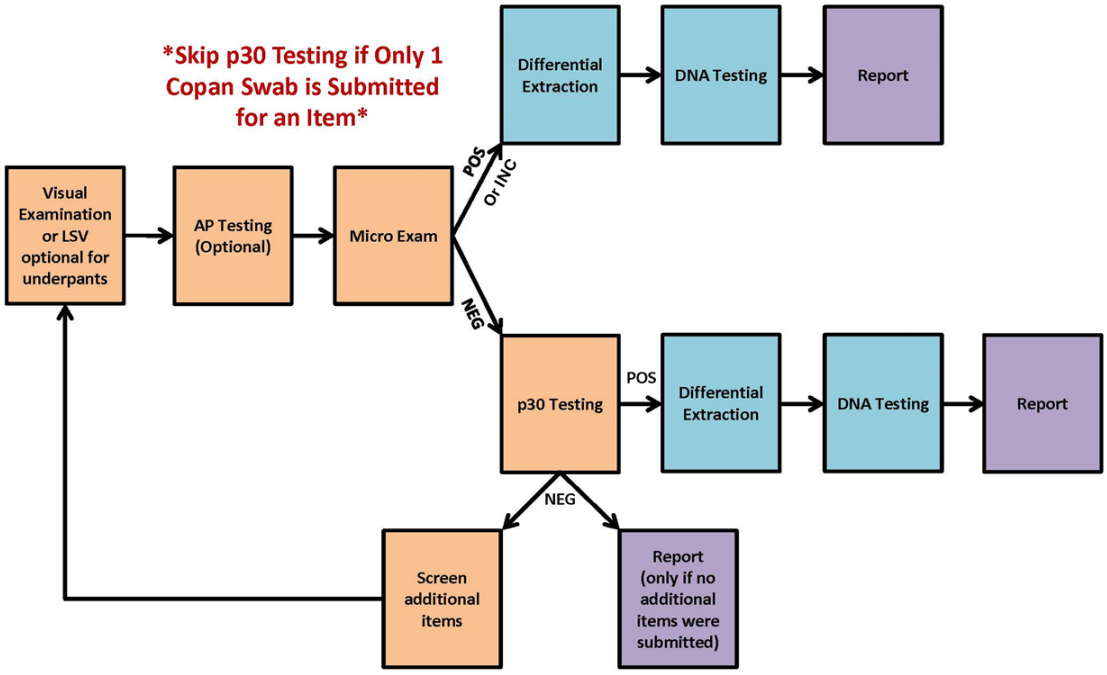
5.3.1.4.5.1.1. Analysts may cut up the sides and lay flat to test.

5.3.1.4.5.1.1.1. If cuts are made, this must be noted. Additionally, the examiner must take pictures of underpants prior to cutting.

5.3.1.4.5.2. If positive, a cutting of the stain will be taken directly to Differential Extraction. If negative, the item can be stopped and reported.

5.3.1.4.5.3. If a swab or a stain (from underpants) will be consumed for testing, a Micro Slide Prep will be created before sampled and consumed.

5.3.1.5. Decision Tree for Screening Swabs, SAECK's, and Underpants (Male Victim/Male Suspect)



5.3.1.5.1. When screening swabs, SAECKs, and /or underpants in investigations involving male victims and male suspects, DNA Quantitation to detect the presence of male DNA is not a useful tool; therefore, the Decision Tree for Screening Sexual Assault Kits (Male Victim/Male Suspect) should be

routinely used. Again, on occasion, circumstances may render deviations from this practice.

5.3.1.5.2. For both nylon-flocked (i.e., Copan) and cotton swabs, AP testing is optional for male victim/male suspect SAECKs.

5.3.1.5.3. For SAECKs in which two nylon-flocked swabs are submitted for a piece of evidence, one swab will be used to prepare the microscope slide. If the micro exam is negative, that same swab will be used for p30 testing. If the p30 exam is positive, the remaining swab will be used for DNA testing. If unable to consume the sample, half of the remaining swab will be used for DNA testing.

5.3.1.5.4. If only one nylon-flocked swab is submitted for a piece of evidence, the swab will be used for the micro exam and potential DNA testing. p30 testing is not performed in order to conserve sample.

5.3.1.5.5. For SAECKs in which cotton swabs are submitted for a piece of evidence, a cutting from one swab, or the entire swab, may be used to prepare the microscope slide. A cutting from the swab used to prepare the microscope slide should be used for DNA testing. For p30 testing, a cutting from the swab used to prepare the microscope slide, or a cutting from the second cotton swab, may be used.

5.3.1.5.6. Regardless of screening results for male victim/male suspect SAECKs, circumstances may arise in which samples are forwarded to DNA testing (i.e., child/ambiguous scenarios). The analyst may choose to forward these samples for either non-differential or differential extraction.

5.3.1.5.7. For SAECKs in which cotton swabs are submitted for a piece of evidence where digital penetration is suspected, a cutting from each swab, equaling approximately one total swab, will be sampled. The analyst may choose to forward these samples for either non-differential or differential extraction. This sampling method can also apply to suspected saliva cotton swabs.

5.3.1.5.8. Underpants should be AP mapped.

5.3.1.5.8.1. Analysts may lay flat and cut up the slides to test.

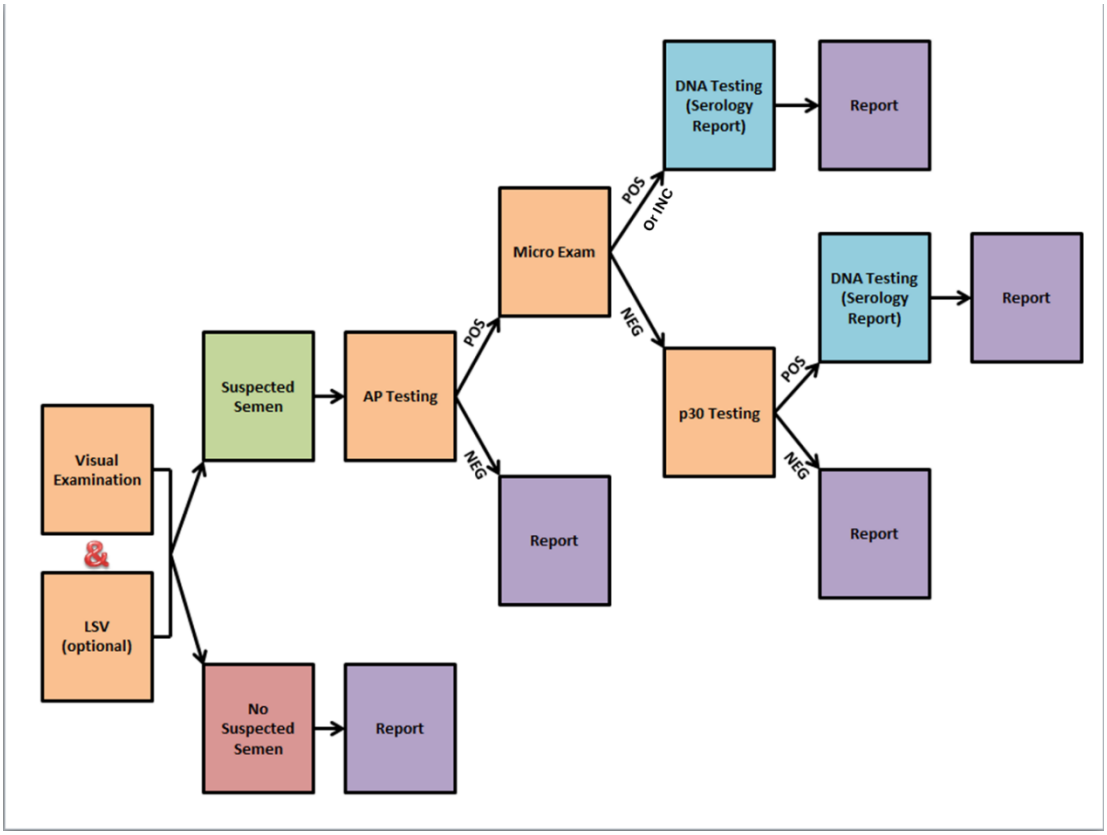
5.3.1.5.8.1.1. If cuts are made, this must be noted. Additionally, the examiner must take pictures of the underpants prior to cutting.

5.3.1.5.8.1.2. If positive, a cutting of the stain will proceed with the testing described in the decision tree above.

5.3.2. Other Suspected Semen Stained Evidence

5.3.2.1. When screening evidence suspected to contain semen that does not fall in the category of swabs, SAECK's, or underpants the Decision Tree for Semen Screening Bulk Evidence should be routinely used to process these sample types; however, on occasion, circumstances may render deviations from this practice.

5.3.2.2. Decision Tree for Semen Screening Bulk Evidence



5.3.2.3. Multiple suspected semen stains are alphabetically identified A, B, D, etc. (C is not used in that the letter is used to indicate a sample is consumed).

5.3.2.4. Condoms and penile swabs do not require routine screening for semen. A swab from each side of the condom should be sampled and processed separately.

5.3.2.5. Bulk items placed inside of sexual assault kits should still follow the Decision Tree for Semen Screening Bulk Evidence, when applicable.

5.3.2.6. When multiple stains test AP positive, analysts can choose to further test all stains or just the stains that they would take forward to DNA testing if there is a

positive micro/p30 result. If the stains the analysts choose test negative, then analysts would continue to test other stains until there is a positive result, or they all test negative.

5.3.2.7. Feminine hygiene products and diapers

5.3.2.7.1.1. Pads in kits and diapers should be treated like a bulk item.

5.3.2.7.1.1.1. If possible, avoid sampling into the absorbent (e.g., gel material) layers.

5.3.2.7.1.2. Tampons should be treated in the same manner as a cotton swab.

5.3.2.7.1.2.1. The examiner should take a cutting from the top, bottom, and center of the tampon and combine in one tube.

5.3.2.8. Prepared Smears

5.3.2.8.1. Smears are not routinely processed. If swabs of the body areas represented by the smears are submitted, the swabs will be processed in lieu of the smears. If swabs of the body areas are represented by the smears, the smears may be processed by swabbing and extracting according to the Differential Extraction Procedure of the MNPD-CL Forensic Biology Technical Procedure Manual.

5.4. Testing

5.4.1. Any serological testing will adhere to the appropriate protocol(s) outlined in the MNPD-CL Forensic Biology Technical Procedures Manual.

6. Other Suspected Body Fluids and Skin Cells

6.1. Body fluids, such as vaginal fluid, saliva, perspiration, and urine may be probative in an investigation. Samples suspected to contain these body fluids will not be screened. The sample will be reserved for PCR-based DNA typing when the presence of these body fluids is believed to be probative. If the case is a sexual assault investigation involving a female victim and male suspect, the Y-screening workflow may be used. Begin the process with non-differential extraction and proceed through the workflow.

6.2. On some items, certain body fluids may be inferred. A reasonable inference would be the presence of vaginal fluid on vaginal swabs or underpants worn by a female. On items such as postage stamps, envelope flaps, chewing gum, cigarette butts, the mouths of bottles and cans, or the mouth opening of face masks, saliva may be reasonably inferred. Finally, on hat bands, the inside of gloves, and cuffs, collars, and bands on clothing a reasonable person may

infer that perspiration and/or skin cells are present. When probative, samples from these items may be PCR-based DNA tested. In some instances, the examiner may use an alternate light source to locate possible body fluids prior to DNA testing.

7. Suspected Tissue

7.1. PCR-based DNA testing may be performed on suspected tissue samples. Blood screening tests may be conducted on substances suspected to be human tissue prior to further processing or solely preserved for PCR-based DNA typing.

8. Hair

8.1. Analysis will be conducted on all appropriate non-hair evidence prior to processing hair. Microscopic hair comparisons will not be conducted by the MNPD-CL Forensic Biology Unit. The probative nature of the information obtained from testing any hair evidence will be carefully evaluated prior to determining the suitability of the hair for nuclear DNA testing.

8.1.1. When evidence is screened for the presence of biological fluids, apparent hair may be collected from the item and/or left on the item for possible future recovery and examination.

8.1.1.1. If apparent hair is collected, the package will be sub-itemized and appropriately labeled and sealed. Forceps should be used to collect the hair. The hair package should be placed in the parent item package. The collection will be reported as “possible hair was collected from this item and returned to the original container”.

8.1.1.2. If apparent hair is not collected and is shed onto the examination paper, return the examination paper in the item packaging.

8.1.1.3. Findings and handling should be documented in the case file (i.e., collection, packaging, observed/not collected, some recovered/some remain, return of examination paper, etc.).

8.1.2. Hair may be submitted previously mounted on microscope slides (i.e., cold cases). Removal of the hair from the slide for PCR-based DNA testing should follow the procedure outlined in the Sample Preparation procedure of the Forensic Biology Technical Procedures Manual.

8.1.3. When hair is selected for nuclear DNA testing, documentation will allow for the tracking of the hair from sampling through DNA interpretation (i.e., sub-item designation when the hair is found on a parent item).

9. Bone and Teeth

9.1. Evidence submitted in the form of bone or teeth may be examined for the presence of suspected soft tissue. Blood screening tests may be conducted on substances suspected to be soft human tissue prior to further processing or solely collected for PCR-based DNA typing. The Forensic Biology Unit will not process bone or teeth samples upon which no suspected soft tissue is found.

10. Sampling Plan

10.1. Once serological examinations have been completed, the evidence can be forwarded onto the DNA analysis process. Other items of evidence not requiring serological screening may move straight into the DNA analysis process. Only a small portion, or sampling, of evidence is generally needed to develop a DNA profile. Using these guidelines, the examiner will sample from evidence requiring processing for DNA analysis. When sampling a piece of evidence by swabbing, a wet/dry swab technique should be performed for trace DNA samples and both swabs processed for DNA.

10.2. Items containing blood: When sufficient sample is present, blood stains will be swabbed for PCR-based DNA typing. In situations, where the suspected blood stain is limited or appears dilute, the analyst may choose to cut the sample from samples that allow for cutting.

10.2.1. CAUTION: Certain porous materials may interfere with bead extraction processes. If DNA results are not consistent with visual observations and/or screening results, swabbing of the sample should be performed.

10.3. Items containing semen: For samples submitted on nylon-flocked swabs, the entire swab head should be cut for PCR-based DNA typing. If multiple cotton swabs are collected from the same source and submitted as a single piece of evidence (i.e., vaginal swabs), a sampling may be processed together in a single tube for DNA.

10.3.1. When sampling non-porous material suspected to contain semen, nylon flocked swabs should be used to collect sample for PCR-based DNA typing.

10.3.2. When sampling porous material suspected to contain semen, an appropriately sized cutting may be taken using a sterile scalpel or scissors to collect sample for PCR-based DNA typing.

10.3.2.1. CAUTION: Certain porous materials may interfere with bead extraction processes. If DNA results are not consistent with visual observations and/or screening results, swabbing of the sample should be performed (See 10.8 of this module).

10.4. Hair evidence: Approximately 1 to 2mm from each end of the submitted hair will be cut and placed into a single tube for DNA extraction. If multiple hairs are submitted as a single evidence item or as a hair standard, a sampling may be cut and placed into a single tube to be taken through the DNA extraction process. A hair should not be sampled unless at least 5cm remain after sampling.

10.5. Items containing saliva: Any non-porous evidence item that could be reasonably expected to contain saliva (i.e., gum, drink bottles, straws, etc.) will be swabbed in the areas that had contact with the mouth using a single sterile swab moistened with autoclaved UltraPure water. The entire swab will be placed into a tube to be taken through the DNA extraction process.

10.5.1. In the instance of porous material (i.e., fabric, cigarette butt, etc.), a cutting may be taken for PCR-based DNA typing.

10.5.1.1. CAUTION: Certain porous materials may interfere with bead extraction processes. If DNA results are not consistent with visual observations and/or screening results, swabbing of the sample should be performed (See 10.8 of this module).

10.6. Stamps and envelope seals: Stamps and envelope seals will be swabbed using a single sterile swab moistened with autoclaved UltraPure water. The entire swab will be placed into a tube to be taken through the DNA extraction process.

10.7. Items for wearer DNA: These evidence items may be examined using an ALS at the discretion of the examiner. Areas of the item potentially containing bodily fluids or prolonged contact areas will be swabbed using swab(s) moistened with autoclaved UltraPure water. The swab(s) will then be placed into a single tube to be taken through the DNA extraction process.

10.8. Items for trace DNA: Items will not be processed for trace DNA if the analyst is aware that item has been previously processed by another forensic unit. When an item requires

forensic processing through multiple units, including trace DNA testing, preservation swabs should be collected from the item, prior to the item being exploited by other forensic disciplines. Any item of evidence that is submitted for trace DNA analysis will be comprehensively swabbed with one sterile swab moistened with autoclaved UltraPure water immediately followed by a comprehensive swabbing with a dry sterile swab. Both swabs will be consumed for DNA by placing into a single tube to be taken through the DNA extraction process.

10.8.1. When receiving a bulk item for trace DNA testing and no information is available about where to test, contact the investigator for clarification. This is needed since there is no screening test available for skin cells.

10.9. Resampling for Re-extraction: When evidence requires re-sampling due to insufficient or no DNA obtained from a porous material, the evidence will be sampled in a different manner than the initial sampling technique (i.e., if initially cut, the sample will be swabbed).

10.10. Reference Standards: When receiving two nylon-flocked or two cotton swabs, take one full swab for DNA testing.

10.10.1. When receiving a single nylon-flocked swab, consume the swab for DNA testing.

10.10.2. When receiving a single cotton swab, cut approximately one half of the swab for DNA testing.

Appendix B Ordering and Receiving

1. Scope

1.1. To describe the process for ordering reagents/consumables/office supplies for the Forensic Biology Unit.

2. Procedure

2.1. Ordering & Receiving

2.1.1. Office Supplies

2.1.1.1. The individual requiring the office supply order will notify the unit procurement designee by email. If a specific item is needed, the email will include the catalog number for the current contracted office supply provider.

2.1.1.2. The unit procurement designee will forward the product description, catalog number, and quantity requested to the crime laboratory Administrative Assistant or Business Manager.

2.1.1.3. Upon receipt, office supplies will be distributed to the requesting individuals or stored in the cabinets of the unit clean area.

2.1.2. Laboratory Supplies and Reagents

2.1.2.1. Vendors of critical reagents and equipment, listed in the QMS, will be evaluated for accreditation or certificate status. Records of evaluations are maintained in the QMS.

2.1.2.2. When laboratory supplies are needed, the unit procurement designee will complete the following steps for ordering:

2.1.2.2.1. Request a quote for the items of interest including shipping via an email to the sales rep of a MNPD approved vendor. This is not required if the vendor provided an open quote.

2.1.2.2.2. Start a “Purchases” workflow in the QMS. The received quote and the completed Form 184 will need to be uploaded in this workflow.

2.1.2.2.3. An order is usually received within 4 – 6 weeks once the workflow is approved by the unit supervisor. During that time, the MNPD Crime Laboratory Business Manager should upload the PO, or purchase order, into the open workflow for that order.

2.1.2.2.4. When an order is received, all items are checked against the Form 184 and shipping receipt. If all items listed on the packing list are received, initial

and date the packing slip. The unit procurement designee will notify the vendor of any discrepancies as soon as possible. Save the shipping receipt in the workflow.

2.1.2.2.5. The shipping receipt and, if applicable, the Certificate of Purity for ISO 18385 certified products are uploaded into the open workflow for that order. This will not be possible until the Business Manager enters the PO information into the workflow. Once the shipping receipt is uploaded, the workflow is complete.

2.1.2.2.6. On supplies that expire, initials, received date, expiration dates, and/or internal lot numbers (composed of an approved item abbreviation and the expiration date) are placed on items received by the person who has received the order. This individual will also ensure commercial reagents are labeled with the identity of the reagent and expiration date. If one is not provided by the manufacturer or distributor, the laboratory will designate the expiration advised by the manufacturer or through research. Check to ensure that the designated lot number does not already exist in the QMS. If so, letter (B, C, D, etc.) may be added to the end of the lot number to differentiate different shipments of the product.

2.1.2.2.7. Next, all items need to be put away in their designated storage areas under manufacturer's suggested storage conditions. Items need to be rotated so that older items or items with shorter expiration dates are moved to the front (i.e., First In First Out).

2.1.2.2.8. Items that expire will be entered into the "DNA Inventory" workflow in the QMS. If quality checks are required, the QMS will automatically launch the appropriate workflow.

2.1.3. Autoclaving and Quality Checks

2.1.3.1. Before use in casework sample processing, the following items will be autoclaved:

2.1.3.1.1. Reagents

2.1.3.1.1.1. Ultrapure water

2.1.3.1.1.1.1. Note: 10% Bleach is made fresh daily with Ultrapure water that is not autoclaved.

2.1.3.1.1.2. 1X TE

2.1.3.1.1.3. Buffer ATL Working Solution

2.1.3.1.2. Consumables

2.1.3.1.2.1. 1.5mL Microcentrifuge Tubes (with the exception of Low-Binding tubes)

2.1.3.1.2.2. 2.0mL Microcentrifuge Tubes

2.1.3.1.2.3. Strip caps

2.1.3.1.2.4. Qiagen 2.0 mL tubes

2.1.3.1.2.5. Qiagen 1.5 mL tubes (flip-top and screw-cap)

2.1.3.1.2.6. Screw caps for Qiagen 1.5 mL and 2.0 mL tubes

2.1.3.1.2.7. 15mL and 50mL conical tubes

2.1.3.1.2.8. 5.0 mL tubes

2.1.3.1.3. Supplies

2.1.3.1.3.1. Tube racks

2.1.3.1.3.2. Conical racks

2.1.3.1.3.3. Tube containers

2.1.3.1.3.4. Screw top Bottles

2.1.3.1.3.5. Glassware

2.1.3.1.3.6. Utensils

2.1.3.1.3.7. Pump Dispensers for Water and TE between Lot Numbers

2.1.3.1.3.8. 96-well Plate racks

2.1.3.2. Before use in casework processing, some reagents will also be quality checked. Please refer to Critical Reagents Quality Control for a complete list of those reagents.

2.1.3.3. The procedure for utilizing the autoclave is outlined in Appendix D of this manual.

3. Safety

3.1. Personal protective equipment shall be worn when entering laboratory spaces.

3.2. Move items to their appropriate laboratory areas as soon as possible. Do not store boxes in hallways or other walkways in the laboratories.

4. Resources

4.1. Forms (sharepoint.com) → Forms

4.2. https://minervabiolabs.us/, for ordering PCR Clean

4.3. http://www.lifetechnologies.com/us/en/home/brands/applied-biosystems, for ordering of consumables for use with AB 7500 and AB 3500 instruments

4.4. http://www.qiagen.com, for ordering of consumables for use with EZ1/2, QIAcube and QIAgility

4.5. http://www.promega.com, for ordering of consumables for use with Plexor, PowerPlex Fusion, and PowerPlex Y23 protocols

4.6. http://www.fishersci.com, for ordering of general laboratory consumables

4.7. http://www.usascientific.com, for ordering of general laboratory consumables

4.8. http://www.kyantec.com, for ordering of general laboratory reagents and chemicals

4.9. http://abacusdiagnostics.com, for ordering of ABAcard® p30 and ABAcard® HemaTrace test devices

4.10. Chemicals FAQ, General Information, http://www.fishersci.com, regarding assigning expiration date if one is not provided by the manufacturer

Appendix C Abbreviations

5CMS	PowerPlex 5C Matrix Standard
6CMS	PowerPlex 6C Matrix Standard
@	at
ABC	anode buffer container
AH2O	autoclaved ultrapure water
AL, LAD	FS allelic ladder
ALS	alternate light source
AMP	Amplification, swab solution amplification solution reagent
AMP'D	amplified
AMT	amount
AN	alpha-naphthyl phosphate
AP	acid phosphatase test
APA	AP spray Solution A
APB	AP spray solution B
APP	apparent
APPROX, ~	approximately
ART	Artifact
AT	analytical threshold
ATL	buffer ATL
AW	AP spray working solution
BB	Fast Blue B
BRN, B	brown
BTW	between

BLD	blood
BH	biohazard
BL	bleach
BP	brown paper
BPB, BPBG	brown paper bag
BPW	brown paper wrap
C	containing, contains, consumed
C5MS	PowerPlex C5 Matrix Standards
CA	capillary array
CBB	cardboard box
CBC	cathode buffer container
CBSM	cardboard slide mailer
CE	capillary electrophoresis
CLPB	clear plastic bag
COLL	collected
CON, CONTR	contributor
CONT	container, containing
CONT'D	continued
CONV	convenience
CPS	Copan swabs
CR	conditioning reagent
CSF	CSF1PO
CTS	Collaborative Testing Services or cotton swabs
CTL	control
CYT	cytoseal

DB	database
D13	D13S317
D16	D16S539
D5	D5S818
D7	D7S820
DCE	debris collection envelope
DE	deduced
Desc	description
DEX	DNA Exitus
DH2O	distilled water
DIFF	differential (extraction)
DIL	dilution
DNA	deoxyribonucleic acid
DTT	dithiothreitol
DNU	data not used, do not use
E-GRAM	electropherogram
E-CELL, EPI	epithelial cell
EDTA	Ethylenediaminetetraacetic acid
E, ENV	envelope
EF	Effersan Tablets
EH	Extraction Hood
ET	evidence tape, lot # designation for EDTA
ETS	evidence tape sealed
ETOH, EL	ethanol
EVID, EV	evidence

EXTE	exterior, external
EXT	extract, extraction
EXT. GEN.	external genital
EZ	EZ1/2 Investigator Kit
FBU	Forensic Biology Unit
FCDNA	FS 2800M control DNA
FLUOR	fluorescence
FLS	Fusion ILS 500
FMM	FS master mix
FN, FOR	Foreign
FNC	fingernail clippings
FNS	fingernail scrapings
FPM	FS primer mix
FR	nuclear fast red
FS	PowerPlex Fusion 5C Kit
FS6C	PowerPlex Fusion 6C Kit
FT	Faint
FWG	FS AMP grade water
FXN	fraction
GAA	glacial acetic acid
GB	bulk G2 buffer
GBK	G2 buffer kit
G/S, GS	glue seal
GL ENV	glassine envelope
GM	GeneMapper IDX

GMS	PowerPlex G5 Matrix Standard
GT	GenTegra-DNA
GTT	gray top tube
HA	Humic Acid
HD	HiDi formamide
HP	hydrogen peroxide
HRS	hours
HS	hospital sticker
HSS	human semen standard
HT	HemaTrace
ID	Identification; Intimate Donor
INIT	initialed
INJ	injection
IFST	Insufficient for further serological testing
ILS	internal lane standard
INC	inconclusive
INFO	information
INT	interior, internal
IP	isopropanol
ITM	intimate
K	known
KM	Kastle-Meyer
LG	large
LT	light, lieutenant
L, LFT	left

LH	left hand
LNC	Letter of Nonconformance
LTT	lavender top tube
LSV	Leeds Spectral Vision
M/, ⓜ	marked (labeled)
M, MAN	manila
MAT	material
ME	medical examiner
MED	medium
MESS	message
MIN	minimum
MJ, MJR	major
MOD	moderate
MFG	manufacturer
MLE	Medical Legal Exam kit
MN, MNR	minor
MR	mixture ratio
MT	methanol
MV	microvariant
N/A	not applicable
NC	not collected
NCON, NOC	number of contributors
NDD	no DNA detected
NE	not examined
NEG, (-)	negative

NOE	not opened or examined
NEP	No exam performed
NFA	no further analysis
NFT	no further testing
NFW	Nuclease-free water
NI	not initialed
NORM	normalization
NR	no results
NS	non-sperm fraction
NT	not tested
NTS	NIST Traceable Samples
NVS	No visible stain
OBS	observed
OL	overloaded or off-ladder allele
OP'D	opened
OWR	owner
PACE	Probabilistic Assessment for Contributor Estimate
PB	paper bag
PCR	polymerase chain reaction
PDC	Plexor HY dye calibration kit
PHY	Plexor HY
PI	picroindigocarmine
PKK	proteinase K in kit
PLB	Plastic bag
PLEB	plastic evidence bag

PERK	physical evidence recovery kit
PHS	PHY DNA standard
PHR	Peak height ratio
PK	proteinase K
PKG	package
PL	plastic
PMK	post mortem kit (medical examiner)
PMM	PHY master mix
PO	POP4 polymer
POS, +	positive
POSS	possible
PPM	PHY primer mix
PQ	PowerQuant system
PQB	PowerQuant Dilution Buffer
PQDC	PowerQuant Dye Calibration kit
PQM	PowerQuant Master Mix
PQP	PowerQuant Primer/Probe/IPC Mix
PQS	PowerQuant Male gDNA Standard
PQW	PowerQuant Amp Grade Water
PRE	Person reasonably expected
PSA, p30	prostate specific antigen, PSA used to designate p30card lot #
PTT	purple top tube
PWG	PHY AMP grade water
Q	question
QC	Quality Control

QMS	Quality Management System
QNS	quantity not sufficient
QUANT	quantitation
RA	Re-amplify
RB	reagent blank
RBD	reagent blank differential (extraction)
RBQ	reagent blank questioned
RBS	reddish-brown stain
RBST	reagent blank standard
RC	EZ1/2 reagent cartridge
REC'D	received
RD	RSID blood
RDE	RSID blood extraction buffer
RDR	RSID blood running buffer
RE	Re-extract
REF	reference
RGD	regarding
RGNT	reagent
RH	right hand
RI	reinject
RN	RNase P plate
RL	reload
RP	replate
RS	random sample
R, RT	right

RTT	red top tube
RW	rework
S, S/	sealed
S/T+I	sealed with tape and initialed
S/T+NI	sealed with tape and not initialed
SAA	sodium acetate, anhydrous
SAT	sodium acetate, trihydrate
SAECK	sexual assault evidence collection kit
SAN PAD	sanitary pad/napkin
SC	stain card
SCH	Sodium Chloride
SDTT	Stock DTT
SF	sperm fraction
SEM FL	seminal fluid
SM	small
SMR	smear
SN#	serial number
Soln	solution
SP	sperm
SRM	NIST Standard Reference Material 2391
SS	staple sealed
SSK	swab solution kit
SSN	social security number
ST, STU	stutter
STD, STND	standard

STER	sterile
STN'D	stained
STO	stochastic threshold
STR	short tandem repeat
SUB	submission
SUSP	suspect, suspected
S/W, SW	sealed with
SW	Swab solution kit swab solution reagent
TA	TrueAllele
TE	tris-EDTA and TE Buffer lot# designation
TR	tris-HCL
TT	test tube
U	unsealed
UP	underpants
UPH2O	ultra pure water
V	very
VICT	victim
V/C	vaginal/cervical
VAG	vaginal
VF	vaginal fluid
VM	voicemail
W/	with
W/D	wet/dry swabs
W/I	within
W, WH	white

WK	weak
WPB	white paper bag
WPW	white paper wrap
WR	Wearer
XYL	xylene
YW	yellow
YWG	YK AMP grade water
YTT	yellow top tube
ZNC	zinc shot

Appendix D Decontamination of Consumables Using the Autoclave

1. Scope

1.1. To describe the process of decontamination of glassware and consumables by autoclaving

2. Equipment/Materials/Reagents

2.1. Tuttnauer Autoclave
2.2. Autoclave tape

3. Procedure

3.1. Wipe inside of Autoclave chamber clean with cloth. If necessary, Ultrapure water and a detergent may be used for cleaning. Do not use bleach products to clean autoclave chamber.
3.2. Load Autoclave with desired items. Programs are designed for unwrapped instruments (1), wrapped instruments (2), glassware/liquids (3), or dry cycle only (4). Consider which program is desired when deciding what can be autoclaved together in the chamber.
3.3. Ensure that autoclave tape is placed on each tray or item within the chamber.
3.4. Allow space between each item so no two surfaces are touching one another or the walls of the chamber.
3.5. All closed containers should have the lids loosened or slightly ajar while autoclave is running.
3.6. When using a paper/plastic autoclave bag, the plastic side should be facing down on the rack.
3.7. Empty canisters should be placed upside-down within the chamber to prevent the accumulation of water.
3.8. Liquids should be sterilized in a heat proof container. The beaker should only be 2/3 filled and the lid should be loosely screwed on.
3.9. Check to ensure the water level of the water reservoir reaches the base of the safety valve. If low, Ultrapure water should be poured though the opening on top of the autoclave until the water reaches a suitable level.
3.10. Select the desired program, specifications of the program will be displayed digitally pertaining to temperature, sterilization time, and dry time. These specifications can be adjusted for each program by using the “up” and “down” buttons, if desired.
3.11. Confirm the door of the autoclave is tightly closed and locked.
3.12. Press “Start” to begin autoclave.
3.13. Upon completion of the autoclave cycle and the chamber has depressurized, the door may be cracked during the drying cycle to allow drying. Once the drying cycle is complete, remove the load from the chamber and ensure that all sterilizing indicators by the autoclave tape had a successful color change.

Appendix E Driftcon® ProbeFixture™

1. Scope

1.1. This Appendix provides instructions on utilizing the Driftcon® ProbeFixture™ to conduct performance checks and maintenance on various instruments in the laboratory.

2. Safety Warnings and Guidelines

2.1. The Driftcon® ProbeFixture™ system is a delicate high-precision temperature measurement instrument and should be handled and operated carefully. The temperature sensors are the most sensitive part and should be treated with special care and attention.

2.2. The temperature sensors may get hot during usage! Take special attention to this when removing the sensors from a thermocycler sample block.

3. Procedure

3.1. Log into the laptop located in the post-amp lab and connect to the wireless internet. Make sure the pathway to the G: drive is active before running the Driftcon. A user logging in to the laptop for the first time will need to login with the Ethernet cable connected to the laptop.

3.2. Log into the CyclerTest website cylertest.com.

3.3. Open the Driftcon software by selecting the Driftcon icon on the laptop's desktop and log in.

3.4. Within the Driftcon software, if the synchronization icon is flashing, it is necessary to update the system with the latest market specifications. Click the SYNCHRONIZE button to begin this process. When synchronization is complete, the Driftcon components can be setup.

3.5. With the laptop plugged into a power source, set up the Driftcon next to the instrument. Plug the USB cable into the back of the Driftcon's hardware box. Connect the other end of the USB to the laptop's USB port. Use the white connection cable, to connect the Driftcon hardware box to the Driftcon's probe-fixture.

3.6. Before data collection, make sure there are enough purchased measurements downloaded on the hardware box by selecting TOOLS->CREDITS. If needed, additional measurements can be requested on-line. For this request, the hardware box serial number is required. After the request a "measurement credit code" will be sent via email. See Driftcon Operations Manual for more information of how to obtain new measurement credits.

3.7. To begin, click the START button to open the 'start wizard'.

3.8. On Page 1, select (q)PCR as the measurement type. Select NEXT.

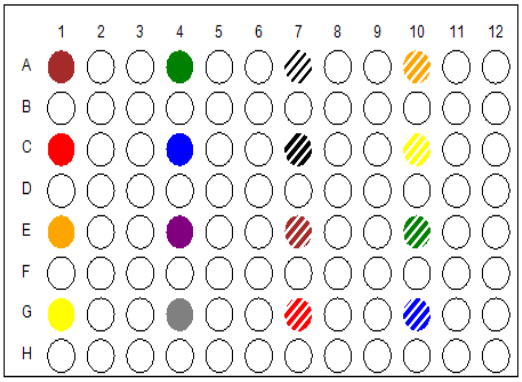
3.9. On Page 2, select the corresponding instrument for calibration. Select NEXT.

3.10. On Page 3, confirm a sufficient number of measurements are remaining. Select NEXT.

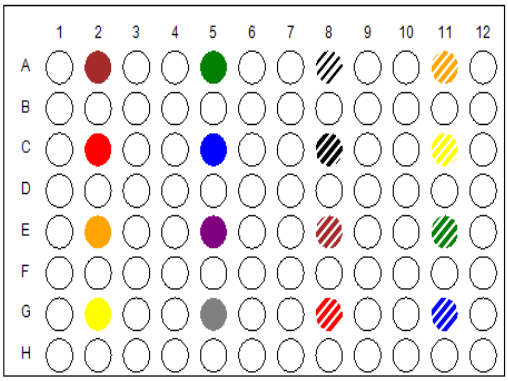
3.11. On page 4, select the DRIFTCON protocol. Select NEXT.

3.12. On Page 5, select the desired layout. Place with probe fixture in the same orientation as the chosen layout. Be sure when placing the temperature sensor within the instrument it lays fully seated inside the wells and the white electronic strip of the probe fixture lays flat within the instrument. The first data acquisition will occur with the top left probe in well A1 (Layout 1). Data collection of temperatures will occur six times per instrument (see layouts below). Select NEXT.

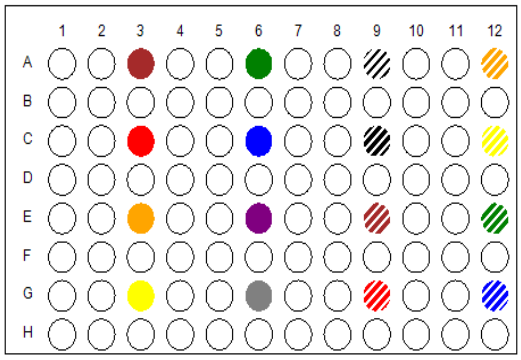
Blocklayout 96v-15 Layout 1



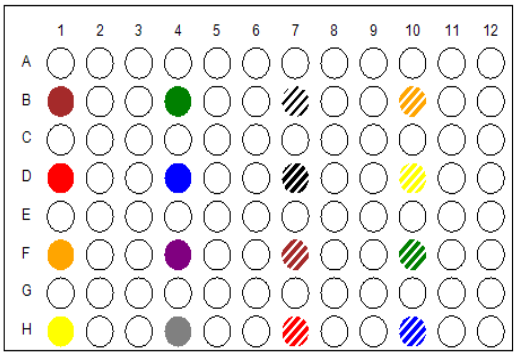
Blocklayout 96v-15 Layout 2



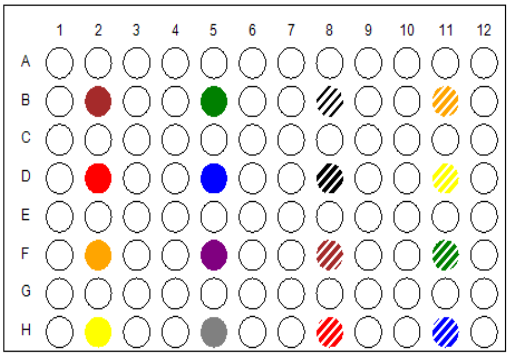
Blocklayout 96v-15 Layout 3



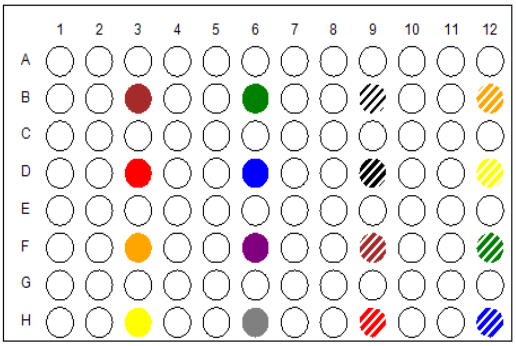
Blocklayout 96v-15 Layout 4



Blocklayout 96v-15 Layout 5



Blocklayout 96v-15 Layout 6



3.13. On page 6, environment conditions can be left blank. Select NEXT.

3.14. On page 7, for the measurement procedures select the control mode to be STANDARD MODE, the reaction volume set at 20, and the heated lid turned OFF. Select NEXT.

3.15. On page 8, comments may be listed, if necessary. Select NEXT.

3.16. On page 9, DO NOT press FINISHED until the Driftcon program has begun on the Veriti Thermal Cycler (go to step 17) or the 7500 instrument (go to step 20).

3.17. For the Veriti Thermal Cycler:

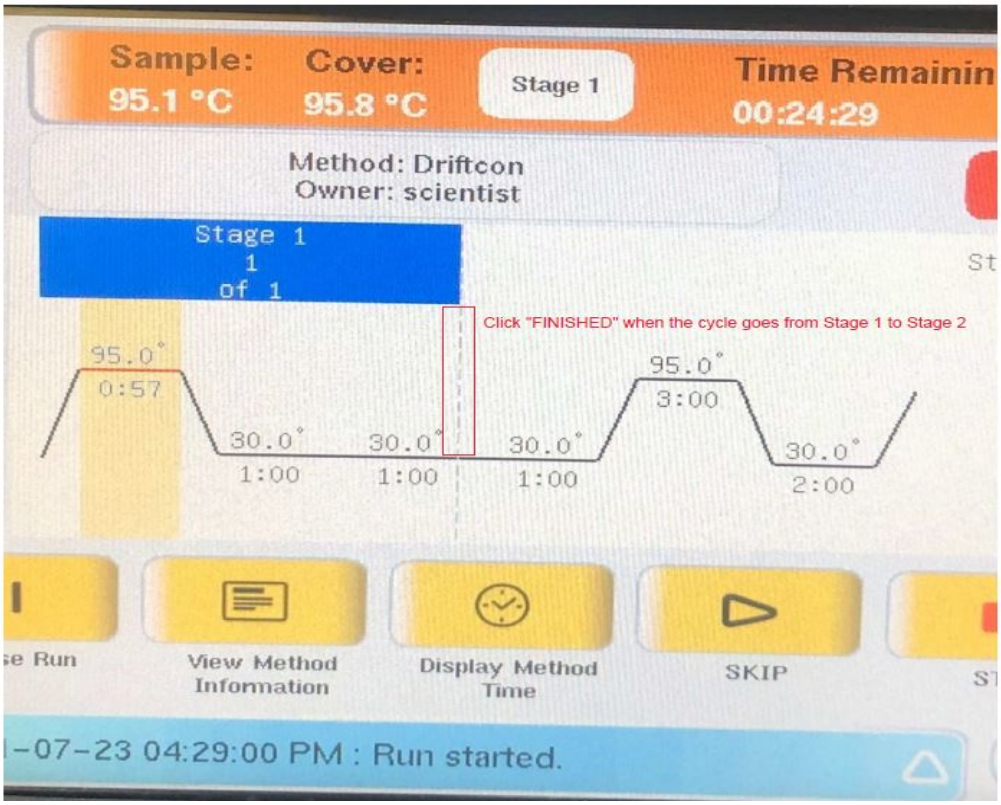
3.17.1. Turn on the Veriti Thermal Cycler and select the protocol Driftcon under the "Browse/New Methods" screen. Make sure "Do not Heat cover" is checked.

3.17.1.1. Note: Heating the cover while the probe-fixture is in the Veriti Thermal cycler could damage the probe.

3.17.2. After verifying the probe fixture is in the correct layout, place the FFC0017 weight on top of the probe to keep it in place.

3.17.3. Leave the Veriti lid open during the Driftcon procedure

3.17.4. Start the Driftcon protocol on the Veriti. When the temperature plot on the Veriti Thermal Cycler goes from Stage 1 to Stage 2 (see photo below), immediately click "FINISHED" within the Driftcon 'start wizard' to begin data collection.



3.17.5. Continue with Driftcon Report.

3.18. For the 7500s Real-Time PCR Instruments:

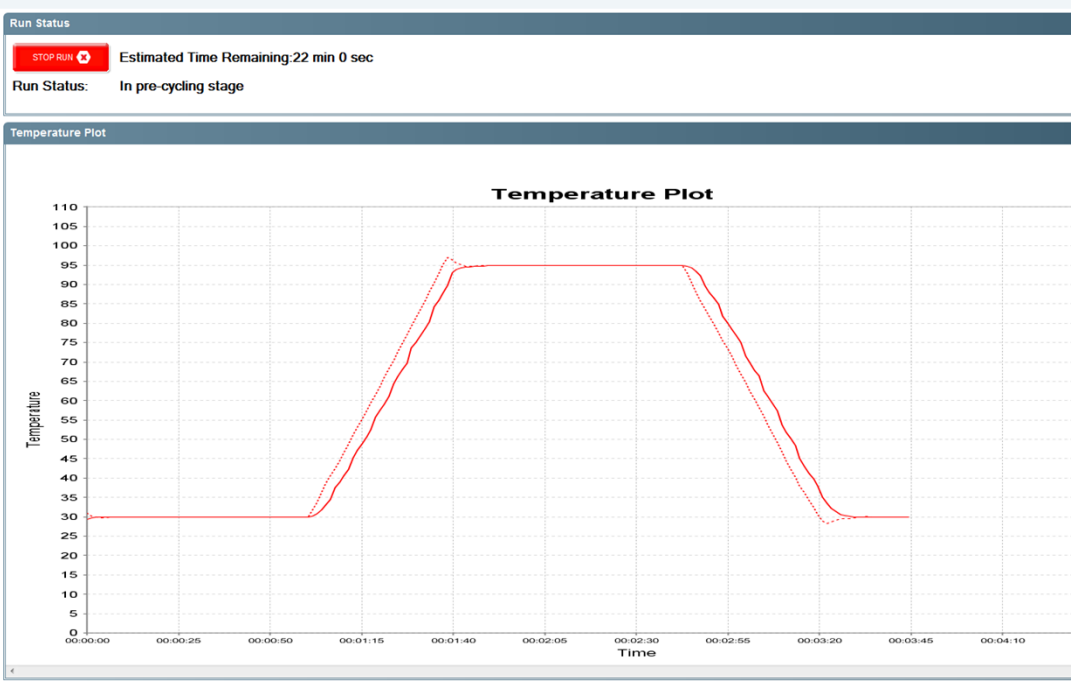
3.18.1. Turn on 7500s instrument and open the HID Real-Time PCR Analysis Software icon on the corresponding computer.

3.18.2. Within the HID software, select NEW EXPERIMENT, FROM TEMPLATE, and then select Driftcon.edt. Once the Driftcon protocol is opened on the 7500 computer, provide an experiment name under SETUP, within EXPERIMENT PROPERTIES.

3.18.3. Select START RUN and save the experiment. This will automatically begin the Driftcon protocol.

3.18.4. View the Temperature plot. Change temperature plot view to read 5 minute increments. Do not select FINISHED within the Driftcon software until the HID Real-time software successfully brings the sample and block to a temperature of 30˚C within the pre-cycling stage (see image below). Once, the two temperature plots converge; immediately click FINSIHED within the Driftcon software to begin the collection of the data by the Driftcon.

3.18.5. Continue with Driftcon Report.



3.19. Driftcon Report

3.19.1. When the Driftcon protocol is complete, preview the report of the Driftcon results generated by the Driftcon software. Ensure all results pass for step, accuracy, and uniformity of each run. If the run does not pass for a particular layout the temperature collection may be repeated. Save all Driftcon reports to the G:drive in the Maintenance Folder. These reports will be uploaded into the QMS.

3.19.2. Complete the 6 layouts on each instrument to ensure all 96-wells are tested. To begin the next run, press the START button again (step 7) and repeat the 'start wizard' of the Driftcon program, ensuring the correct layout is chosen Repeat the procedure until all 6 layouts are complete.

4. Reference

4.1. Driftcon Operations Manual, Version 2.0, 2017. https://www.cyclertest.com/documentation/driftcon/manual.aspx

Appendix F SAMS-Track

SAMS-Track is a sexual assault kit tracking system that was implemented in compliance with TCA 38-6-128 and TCA 39-13-519, which was passed in 2021 by the Tennessee General Assembly. The tracking system provides sexual assault victims with the ability to anonymously track the location and status of their kit from the point of collection through forensic analysis to final storage location and possible destruction. When processing a sexual assault kit, check the collection date on the kit paperwork. All kits collected after July 1, 2022, must be tracked in SAMS-Track.

1. Requirements

1.1. Per TCA 38-6-128 and TCA 39-13-519, several deadlines are set in place from collection to disposition of the sexual assault kit:

1.1.1. Medical facility must notify law enforcement within 24 hours of the forensic examination.

1.1.2. Law enforcement must pick up the sexual assault kit within 7 days of the notification.

1.1.3. Law enforcement must enter the kit into the system within 10 days of picking up the kit from the medical facility.

1.1.4. Law enforcement must submit the kit to the laboratory within 30 days of taking possession.

1.1.5. If the victim elects not to report the alleged offense at the time of the examination, the SAECK becomes a hold kit. Hold kits must not be submitted to the laboratory for testing. If received, call the submitting officer and return the kit.

1.1.6. Hold kits that are released by the victim may be submitted to the laboratory for testing. Kits can be held with the agency for up to 10 years. At MNPD, hold kits will be stored at the Evidence Storage Division (ESD).

1.2. The FB unit is responsible for ensuring that the appropriate milestones pertaining to the examination of the kit are documented in SAMS-Track. See Edit a SAFE kit section.

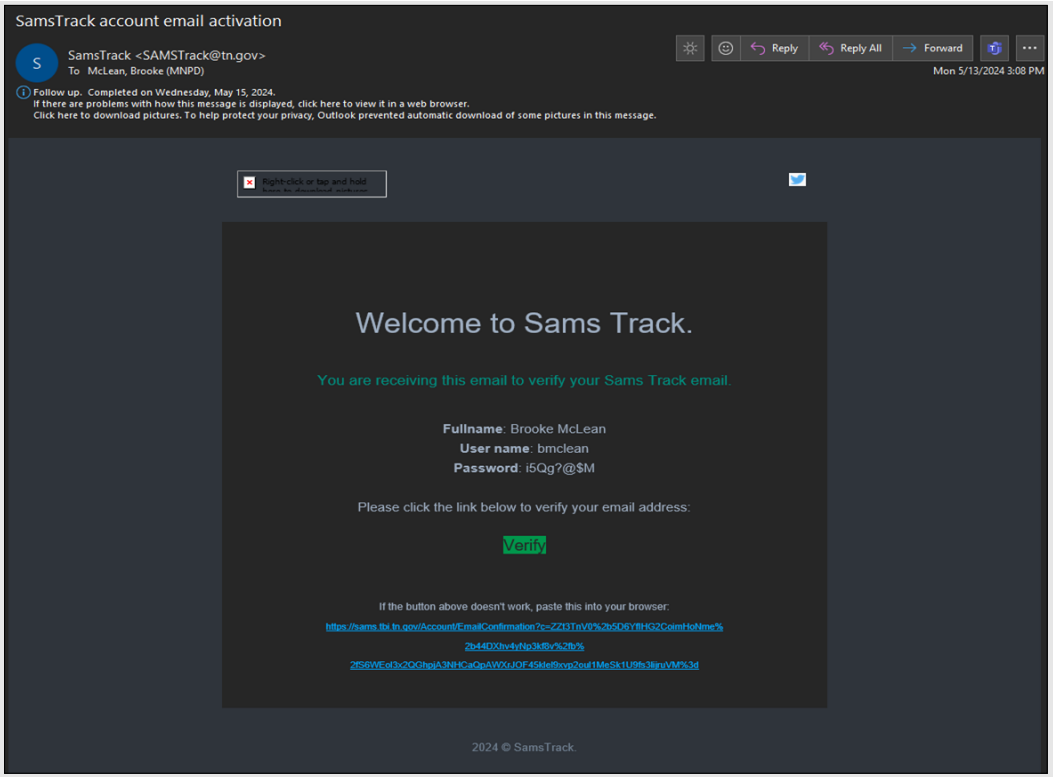
2. Activate your Account

2.1. Email supportsamstrack@tn.gov with your first and last name, email address, phone number, the name of your organization, and the judicial district where you work. This

information will be used to add a user to SAMS-Track. Once added, you will receive an activation email.

2.2. Check your email for a message from SAMS-Track.

2.3. Open the activation email.



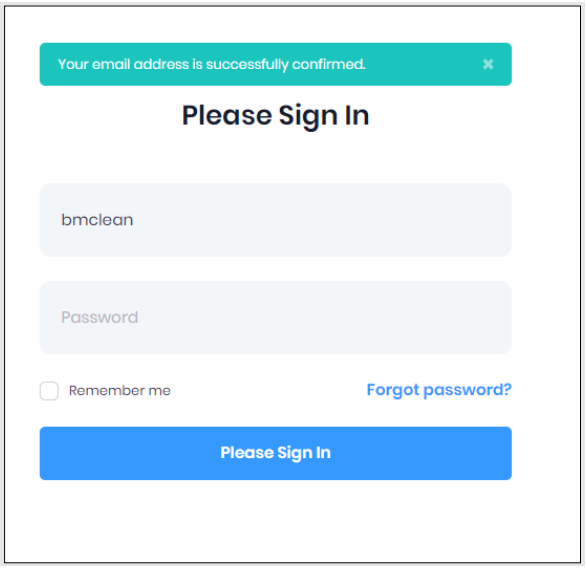
2.4. Copy the temporary password from the email.

2.5. Select the green verify button.

2.6. Enter your email address or username.

2.7. Enter your temporary password.

2.8. Select “Please Sign In”



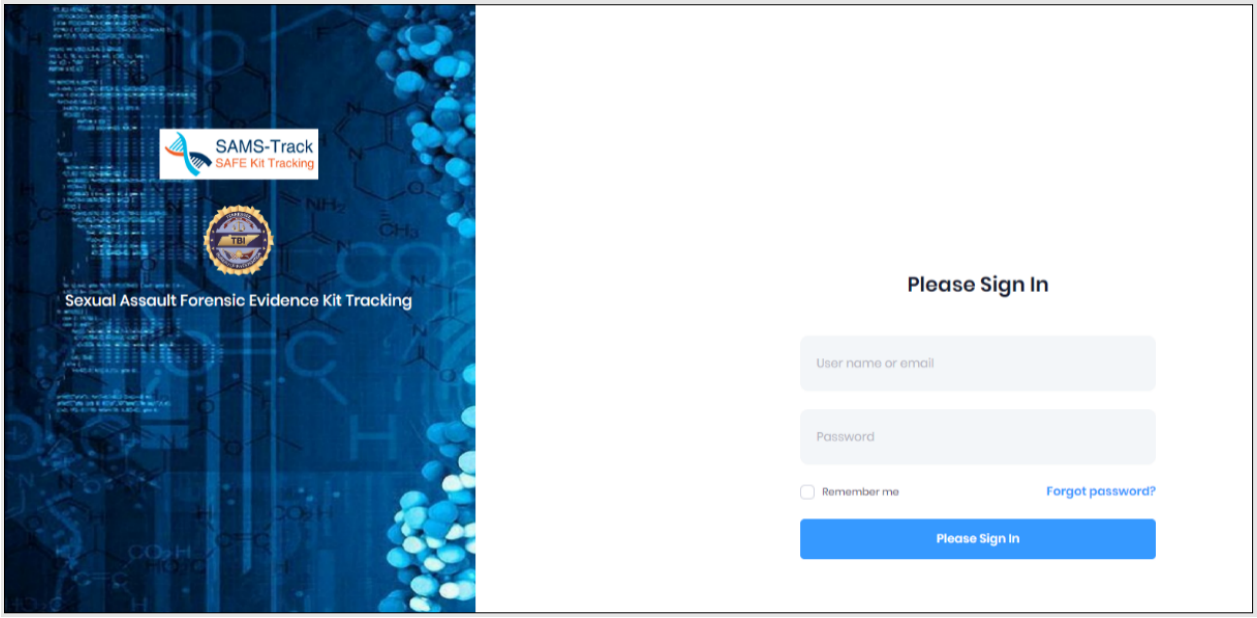
2.9. Enter a new password twice to replace the temporary password.

2.10. Select “Submit”. The account is activated, and you will be logged in.

3. Log in to your Account

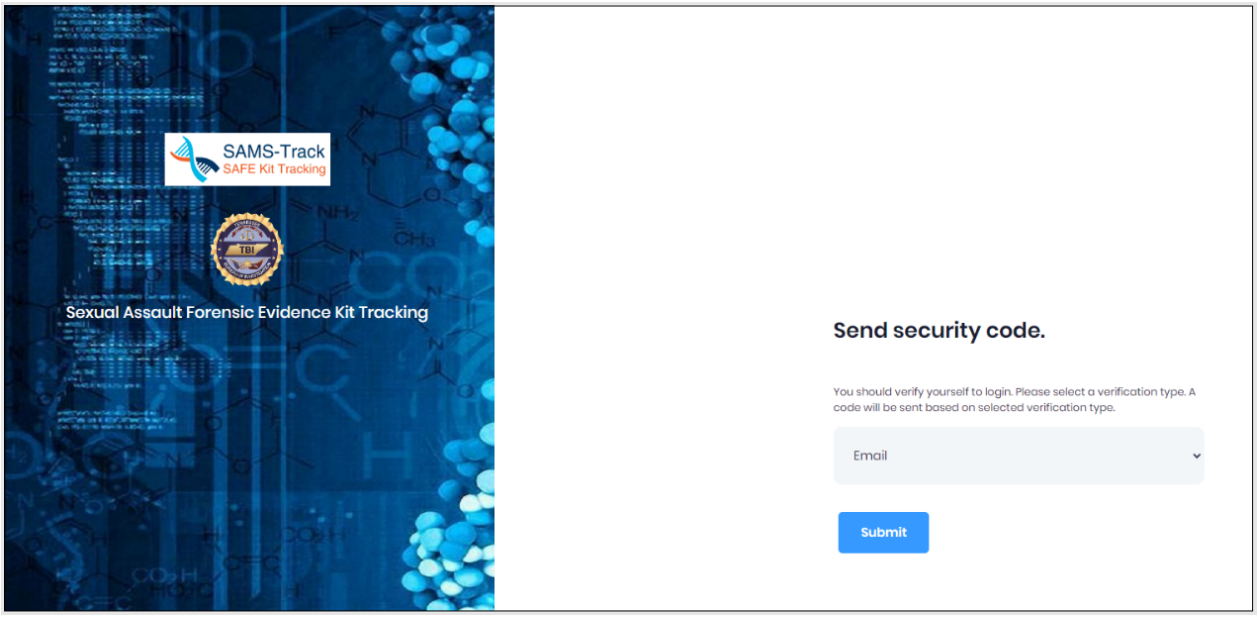
3.1. Go to SAMS-Track at https://sams.tbi.tn.gov/Account/Login

3.2. Enter your username or email address and password.



3.3. Select “Please Sign In”

3.4. “Send security code” will appear with “email” in the dropdown menu. Select “submit” and enter the security code sent to your email address.

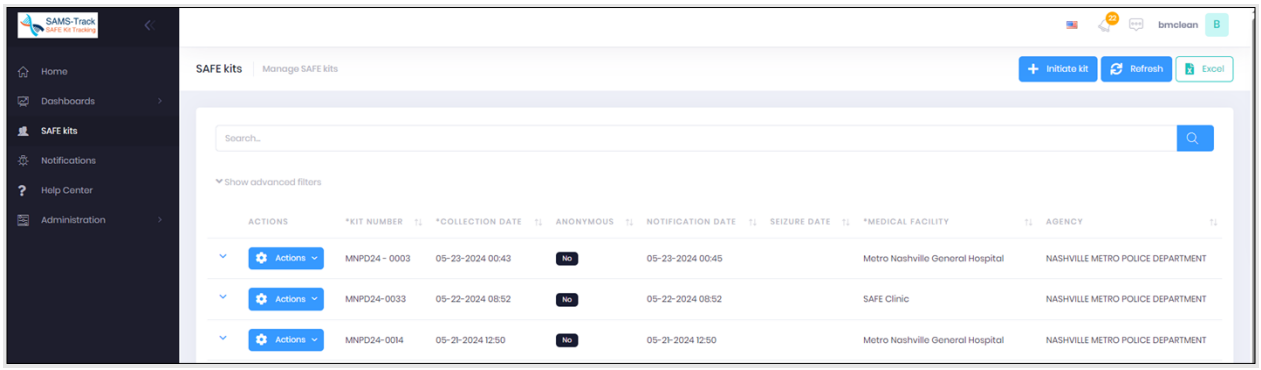


4. View a SAFE kit (Sexual Assault Forensic Examination kit)

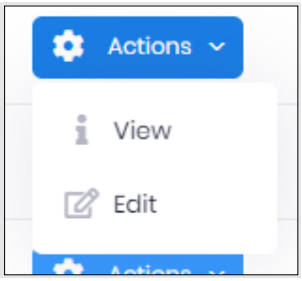
4.1. 1. Select the “SAFE kits” button on the left side menu and use the search bar to find the kit.

4.1.1. MNPD-FBU has created a naming scheme to identify SAMS-Track IDs on paperwork, outer packaging, and/or other documentation. This includes the agency and year, along with the kit number (e.g., MNPD22-0001).

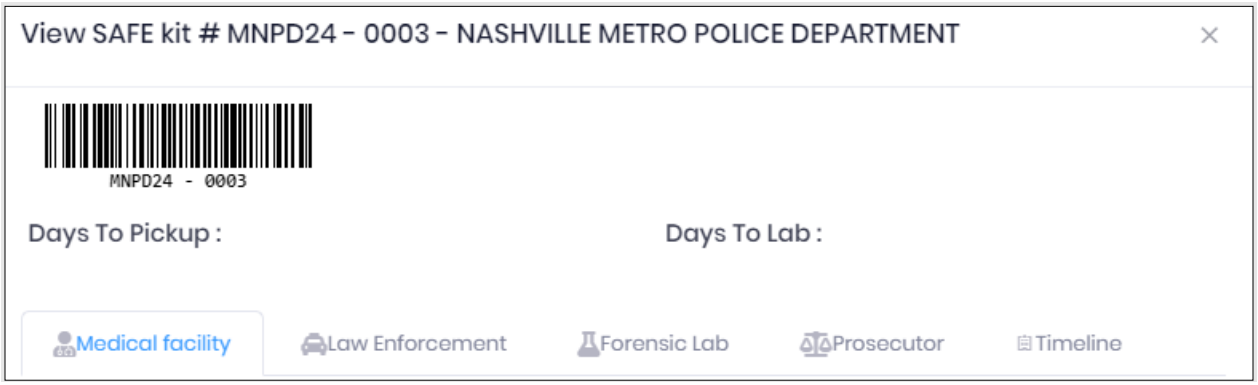
4.1.2. Alternative tracking numbers can be used by medical facilities and might not be clearly marked as a SAMS-Track ID. If unable to locate a SAMS-Track ID, enter the agency/incident number into the search bar to locate the kit.



4.2. Select the blue “Actions” button next to the kit and select “View”.



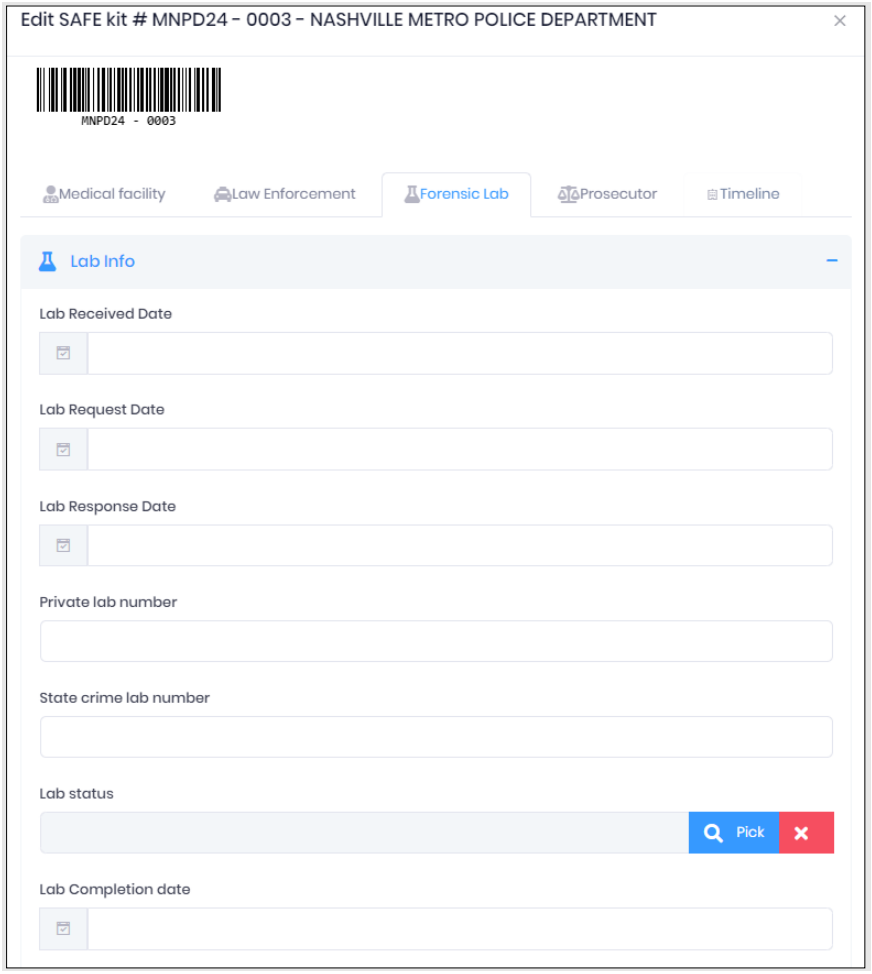
4.3. View kit information by selecting one of the following tabs: Medical Facility, Law Enforcement, Forensic Lab, Prosecutor, Timeline.



5. Edit a SAFE kit

5.1. Select the “SAFE kits” button on the left side menu and use the search bar to find the kit.

5.2. Select the blue “Actions” button next to the kit and select “Edit”. FB personnel are only able to edit information within the “Forensic Lab” tab.

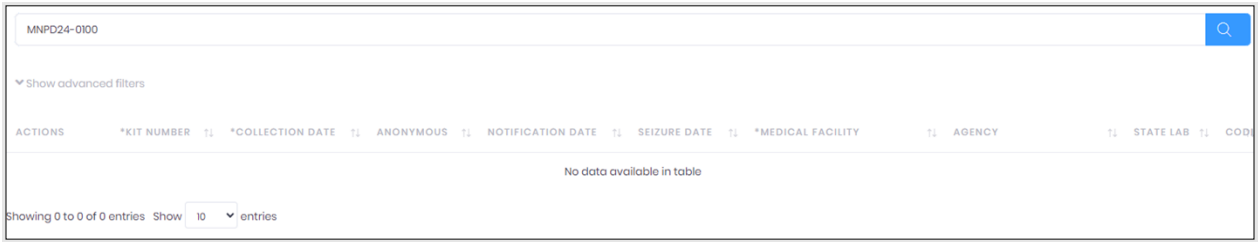


5.3. Lab Status: Select “In-Process” once examination of the kit has started.

5.4. Lab Status: Select “Completed” after completion of administrative review. Add the completion date below the “Lab Status” box.

5.5. Select “Save” to save changes.

6. SAFE kit not found in SAMS-Track



6.1. If a SAECK has a SAMS-Track ID, but has not been entered into SAMS-Track, first contact the investigator and request that they add the kit in the software. If the kit has not been added after contacting the investigator, follow the steps below to initiate the kit in SAMS-Track.

6.1.1. Select the blue button at the top right of the SAFE kits tab "+ Initiate Kit"

6.1.2. In the Medical tab, enter*:

6.1.2.1. Kit Number: SAMS-Track ID or Agency #

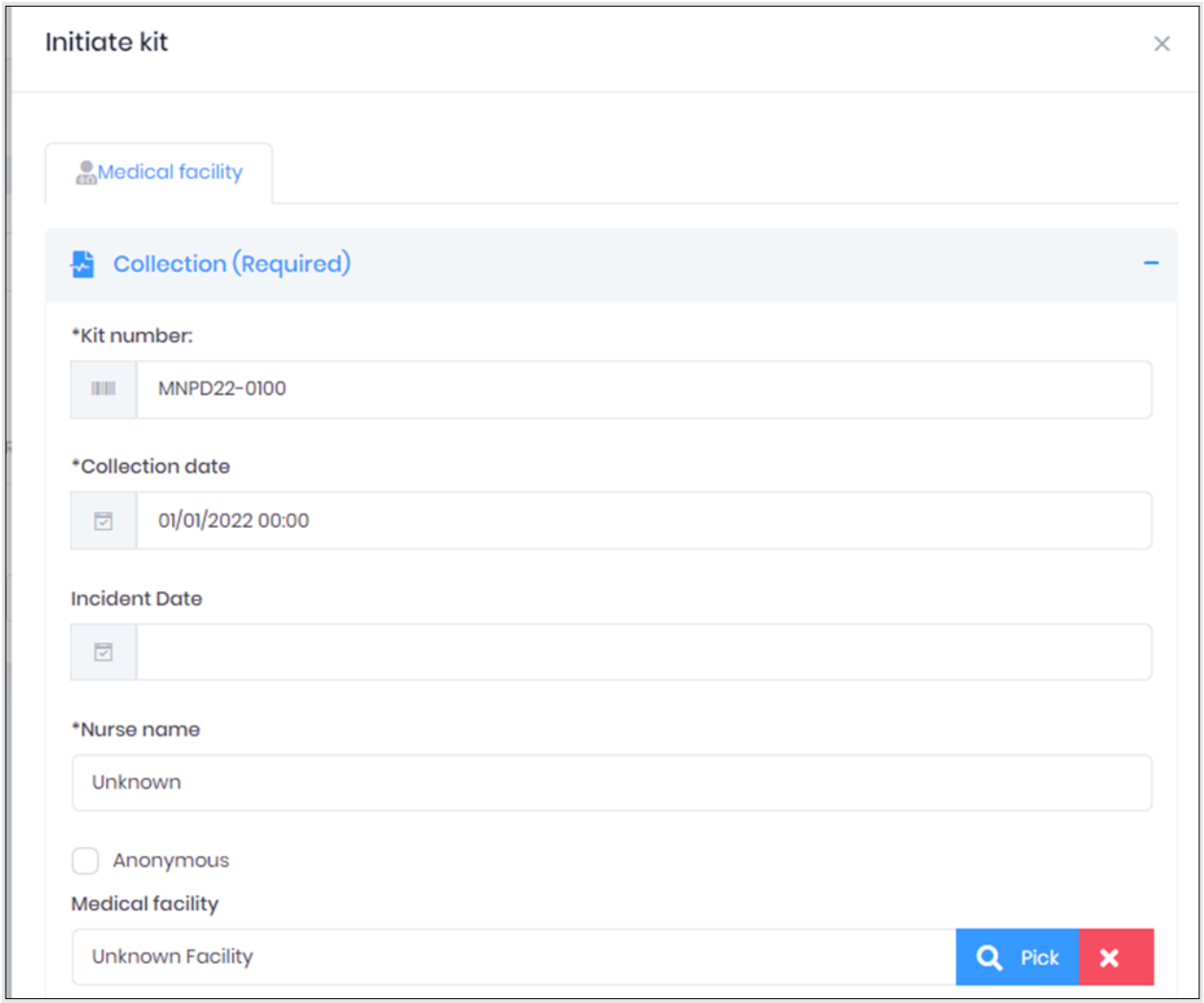
6.1.2.2. Collection Date: "1/1/2022"

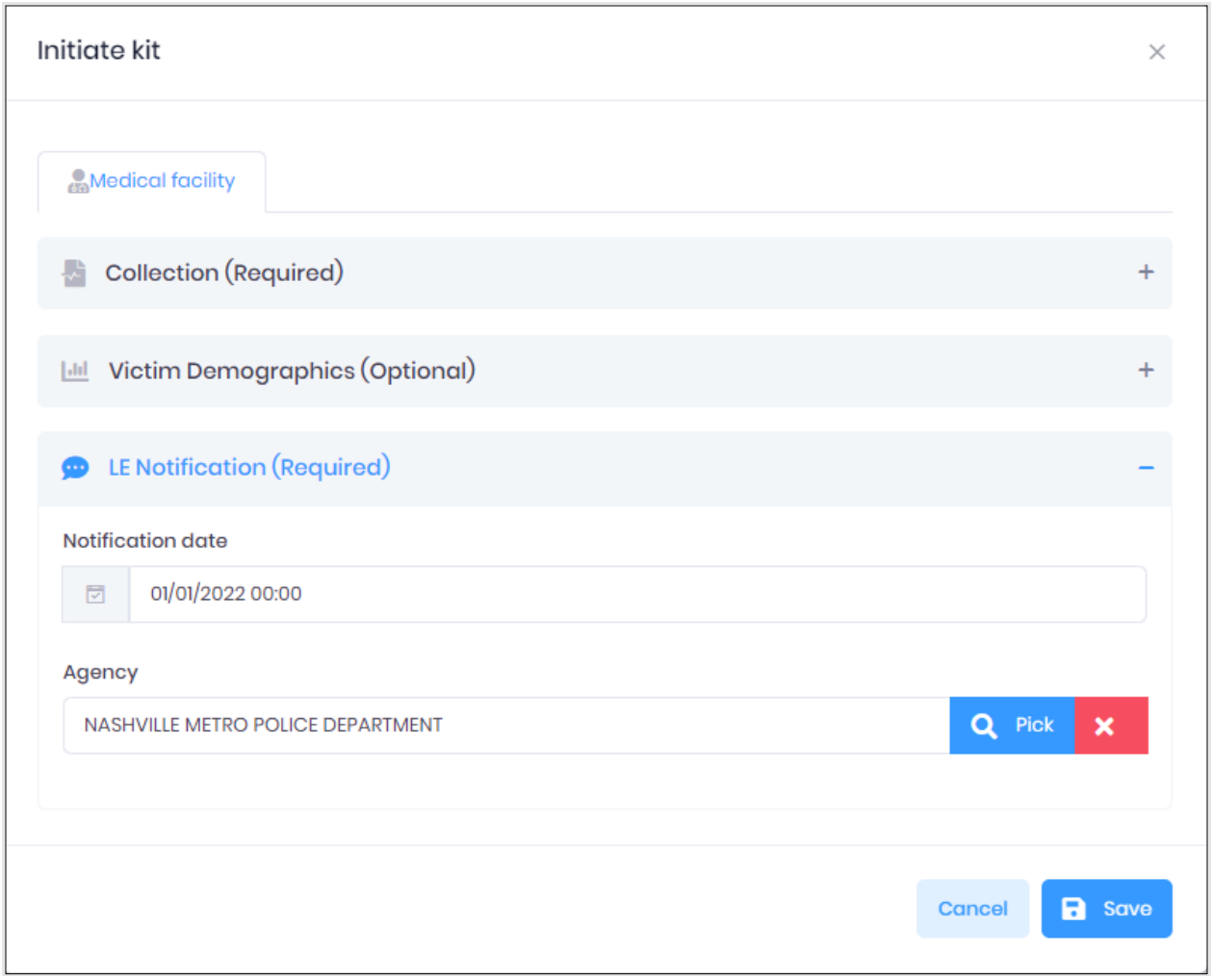
6.1.2.3. Nurse Name: "Unknown"

6.1.2.4. Medical Facility: "Unknown Facility" (use dropdown menu)

6.1.2.5. LE (Law Enforcement) Notification Date: "01/01/2022"

6.1.2.6. Agency Name: Use dropdown menu





6.1.2.7. *Use the above info as a default. Upon viewing the kit paperwork, the scientist can backfill this data with the correct information.

6.1.3. In the Forensic Lab tab, enter:

6.1.3.1. Date Received

6.1.3.2. Lab Status: Select “Received”

6.1.3.3. Select “Save” at the bottom right of the screen.

6.1.3.4. Proceed with steps outlined in Edit a Safe kit section.

Appendix G Contamination Database Sample Processing

The following provides guidance to analysts when performing sample processing for the purpose of entry to the lab's contamination database (i.e., GMID-X profile manager and LDIS). Samples collected after 1/15/2026 must be processed and uploaded to the lab's contamination database within 60 days of collection. The instruction below is not intended to be used for processing of casework samples.

1. Initial Extraction
   - 1.1. Initial amplification
     - 1.1.1. If the profile developed has any loci with a single allele below the stochastic threshold and/or observable dropout (partial or complete) the sample must be reamplified at a higher amplification target.
   - 1.2. Reamplification (> initial template)
     - 1.2.1. If the profile developed has ≤ 3 loci below the stochastic threshold and/or observable dropout (partial or complete) these loci will be entered to LDIS as partial.
     - 1.2.2. If the profile developed has > 3 loci below the stochastic threshold and/or observable dropout (partial or complete) these samples must be re-extracted.

2. Re-extraction
   - 2.1.1.2.1 Initial amplification of re-extractionIf the profile developed has any loci with a single allele below the stochastic threshold and/or observable dropout (partial or complete) the sample must be reamplified at a higher amplification target.
   - 2.2. Reamplification of re-extraction (> initial re-extraction template)
     - 2.2.1. If the profile developed has ≤ 3 loci below the stochastic threshold and/or observable dropout (partial or complete) these loci will be entered to the LDIS as partial.
     - 2.2.2. If the profile developed has > 3 loci below the stochastic threshold and/or observable dropout (partial or complete), consult with the CODIS admin.

3. Profiles developed from multiple amplifications/injections can be combined to enter the most complete DNA profile into the LDIS database.

4. Once the profile has been reviewed and uploaded into the GMID-X Profile Manager and the LDIS, the extract and remaining buccal swab(s) should be disposed of.