Procedure for DNA Reagent Preparation and Quality Control

1.0 Purpose - This procedure specifies the required elements for the preparation of, and quality control procedures for, reagents used in the Forensic Biology Section.

2.0 Scope – This procedure applies to all Forensic Scientists in the Forensic Biology Section.

3.0 Definitions – See Section Definition list

4.0 Equipment, Materials and Reagents

- pH test strips (see Forensic Biology Section Procedure for Body Fluid Unit Quality Control)
- Chemicals: Buffer ATE, Buffer ATL, TE buffer
- Nuclease-free distilled water (nuclease-free dH2O)
- Distilled water (dH2O) from in-house filtered water supply system
- Certified Biosafety Cabinet and/or certified chemical fume hood
- Various lab equipment (lab tape, autoclave tape, Alconox (or equivalent), Kimwipes, pipettes and associated tips, cleaned and sterilized glassware, heat/stir plate, vacuum pump, magnetic stir bars, 96-well trays and septa, amplification trays, pH buffers)

5.0 Procedure – All documentation of quality control checks shall be approved by the Technical Leader prior to use in casework.

5.1 NIST SRM/ Standard Traceable to NIST

5.1.1 Purpose and Use: The QCO shall test the analytical procedures used against the appropriate National Institute of Standards and Technology (NIST) Standard Reference Material (SRM), or Standard Traceable to NIST (NIST-TS), on an annual basis. The NIST SRM or NIST-TS shall also be tested when substantial changes, new procedures, or new platforms are validated in these units, as well as against commercially produced kits.

5.1.2 Creating a Standard Traceable to NIST: The QCO shall create a batch of known human bloodstains from a male individual whose DNA profile has been previously established as follows:

5.1.2.1 Dispense liquid blood from donor onto several sheets of FP705 paper (or equivalent) until all collected liquid blood is deposited and allowed to dry completely.

5.1.2.2 A sample from this batch of bloodstains shall then be extracted (using current Forensic Biology Section extraction procedure(s)) along with an associated negative extraction control (Neg K), as well as any part of the NIST SRM that requires extraction (i.e., SRM's E and F in NIST SRM 2391c).

5.1.2.3 This extracted bloodstain and Neg K, as well as any extracted NIST SRMs shall then be quantitated, amplified, electrophoresed and analyzed simultaneously, along with any NIST SRMs which may have already been supplied in liquid form (i.e., NIST SRMs A-D in NIST SRM 2391c) according to applicable Forensic Biology Section DNA procedures.

5.1.2.4 The bloodstain and all NIST SRMs shall provide the expected allele calls, and all testing negatives (including Neg K) shall be free of any alleles. If either condition is not met (for reasons other than instrument failure or known artifacts), then the QCO may retest the bloodstain and/or NIST SRMs once. If both conditions are not met this second time, a new lot of bloodstains and/or NIST SRM shall be tested.

5.1.2.5 Once the conditions in **5.1.2.4** are met (i.e., the expected allele calls are obtained and the testing negatives are free of any alleles), this batch of bloodstains shall be accepted as a suitable NIST-TS and the entire lot of bloodstains shall be named/referred to by the initials of the blood donor, followed by the date on which the bloodstains were prepared (e.g., XXX_12012010). The QCO shall document the testing performed and retain such documentation in the Section, along with the NIST SRM documentation provided by the manufacturer.

5.1.2.6 If other testing kits become available for use in the Forensic Biology Section, the appropriate NIST SRM for that kit shall be tested against a batch of known human bloodstains from a male individual. This batch may be the same NIST-TS currently in use if enough of that batch remains available for testing.

5.1.3 **Storage:** The NIST SRM shall be stored long-term at -20 °C with limited access by the QCO; the NIST-TS (bloodstains) shall be stored with limited access by the QCO at room temperature; extracted NIST-TS (liquid form) and associated Neg K shall be stored at 4°C for up to 1 year after date of approval for use by the DNA Technical Leader with limited access by the QCO for use in QC testing. After 1 year, these extracts shall be discarded by the QCO.

5.2 UV Treatment

5.2.1 Reagents prepared in house shall be treated with UV light exposure for a minimum of 30 minutes after autoclaving prior to use.

5.2.2 Plasticware, to include the tubes used for extraction and quantification and amplification setup, strip tubes, conical tubes for reagents, and 96 well plates used for amplification shall be treated with UV light exposure for a minimum of 30 minutes prior to use. Plasticware used in the post-amp lab does not need to be treated with UV. 96 well plates used for quantitation setup shall not be treated with UV light. Plasticware that has been treated by the manufacturer to substantially reduce the possibility of exogenous DNA (e.g., EO treatment or QC tested for DNA contamination) does not need to be treated with UV.

5.3 Preparation and QC of Reagents/Solutions/Standards – The expiration date provided by the manufacturer will be used for any reagent received within the Forensic Biology Section.

5.3.1 Naming/Recording of Reagents/Solutions/Standards:

5.3.1.1 The following items shall be recorded in FA under the Resource Manager by the QCO as follows: Item lot number expiration date (e.g., A9815D0209_03152011):

- ProK (aliquots)
- DTT (aliquots)
- Formamide (aliquots)
- Carrier RNA (aliquots)
- Nuclease-free dH2O
- Diluted Buffer ATL
- Any item listed in **5.3.1.1** or **5.3.1.2** if purchased directly from manufacturer

5.3.1.2 The following items shall be recorded in FA under the Resource Manager by the QCO based upon the lot numbers provided by the manufacturer. Any expiration dates (if applicable) shall be noted within the individual lot Resource Instance Details:

- Kits (**PowerPlex® Fusion 6C and PowerPlex® Y23**, Quantifiler® Trio, DNA Investigator, Casework Direct, Bone DNA Extraction Kit)
- Kit components (e.g., reaction mix, primer, Taq, DNA standard, allelic ladder, positive and negative amplification controls, Carrier RNA, ProK, Buffer ATL, Buffer ATE, Casework Direct Reagent, 5X Amp Reagent, Demineralization buffer, Lysis buffer)
- 3500XL POP-4, anode and cathode buffers
- ProK, DTT, Hi-Di formamide, nuclease-free dH2O (stock)
- Buffer ATL
- Buffer MTL
- TE buffer

5.3.2 For all items which require testing for reliability (QC check), the date on which the item passes Quality Control (QC) shall be entered into FA under the "date verified" line by the QCO performing the QC check.

5.3.3 Aliquots of differing reagents (e.g., ProK, DTT, cRNA) must be stored in different colored tubes for easy identification.

5.3.4 **Documentation:** Any documentation generated from the preparation or QC check of any reagents, kits or standards shall be documented by the QCO in the QC files and thereafter maintained in the Section.

5.3.5 **Solution/Reagent/Standards Preparation and QC (as noted):**
Note: Glass bottles used in the preparation and storage of buffers and components shall be cleaned with Alconox (or equivalent), rinsed with dH2O and autoclaved prior to use (see Forensic Biology Section Procedure for Aseptic Technique and Contamination Control).

5.3.5.1 0.39M STR-Dithiothreitol (DTT)

Chemical/Reagent	Amount (for 10 mL)	Amount (for 25 mL)

Dithiothreitol (McCleland's reagent, stock)	601 mg	1.5 g
Sterile nuclease-free dH2O	10 mL	25 mL

5.3.5.1.1 Add water to DTT to reconstitute and mix well.

5.3.5.1.2 Aliquot 200 µL into sterile colored 0.5 mL tubes while under a Biological Safety Cabinet (or equivalent).

5.3.5.1.3 Freeze aliquots immediately at -10 °C. Once aliquot is thawed it shall not be refrozen and after use, the remainder of the aliquot shall be discarded. The master supply of aliquots shall be stored at -20 °C; working stock supplies of DTT shall be kept at -10 °C.

5.3.5.1.4 See **5.3.1** for naming convention and FA entry.

5.3.5.1.5 Aliquots expire 1 year after date of reconstitution, or when stock supply expires, whichever occurs first.

5.3.5.2 Hi-Di Formamide

5.3.5.2.1 The QCO shall thaw formamide and aliquot as follows [(0.5 µL WEN ILS 500) x (# of samples)] + [(9.5 µL Hi-Di formamide) X (# samples)] into autoclaved clear 1.5 mL sterile tubes for casework. Aliquots and WEN amounts may be adjusted to account for pipetting variations.

5.3.5.5.2 The aliquots shall be frozen immediately at -10 °C. Once aliquot is thawed it shall not be refrozen, and after use the remainder of the aliquot shall be discarded by the Forensic Scientist. Aliquots expire 1 year after date of preparation, or when stock supply expires, whichever occurs first.

5.3.5.5.3 See **5.3.1** for naming convention and FA entry.

5.3.5.3 Diluted Buffer ATL

Chemical/Reagent	**Amount**
Buffer ATL	**600 mL**
TE Buffer	**300 mL**

5.3.5.3.1 Measure out 600 mL of Buffer ATL

5.3.5.3.2 Measure out 300 mL of TE buffer and add it to the Buffer ATL.

5.3.5.3.3 Mix thoroughly until in solution.

5.3.5.3.4 Place the solution in a labeled bottle.

5.3.5.3.5 Diluted Buffer ATL shall be stored at room temperature and discarded 6 months after dilution or on the date the Buffer ATL or TE buffer expires,

whichever is earlier. If the diluted Buffer ATL falls out of solution, then the diluted Buffer shall be kept at 37 °C, including any aliquots, to keep the buffer in solution.

5.3.5.3.6 QC Testing:

5.3.5.3.6.1 A sample of the NIST-TS (or known DNA standard from a previously typed individual) and Neg K shall be extracted, quantitated, amplified, electrophoresed, and analyzed according to applicable Forensic Biology Section DNA Procedures.

5.3.5.3.6.2 The new lot of diluted Buffer ATL shall be used at the extraction step.

5.3.5.3.6.3 The expected results for the NIST-TS (or known standard) shall be obtained for all loci and the alleles shall be balanced within and between loci and peak heights above the analytical threshold and <15000 RFUs. The Neg K shall be free of any alleles. If either condition is not met (for reasons other than instrument failure or known artifact), the QCO may retest the new lot of Buffer ATL only once. If either condition is not met this second time, the Buffer ATL shall not be accepted for any use in the Section and the DNA Technical Leader and manufacturer shall be notified immediately by the QCO.

5.3.5.3.6.4 See **5.3.1** for naming convention and FA entry.

5.3.5.4 Buffer MTL

5.3.5.4.1 QC Testing:

5.3.5.4.1.1 A sample of the NIST-TS (or known DNA standard from a previously typed individual) and Neg K shall be extracted, quantitated, amplified, electrophoresed, and analyzed according to applicable Forensic Biology Section DNA Procedures.

5.3.5.4.1.2 The new lot of Buffer MTL shall be used at the extraction step.

5.3.5.4.1.3 The expected results for the NIST-TS (or known standard) shall be obtained for all loci and the alleles shall be balanced within and between loci and peak heights above the analytical threshold and <15000 RFUs. The Neg K shall be free of any alleles. If either condition is not met (for reasons other than instrument failure or known artifact), the QCO may retest the new lot of Buffer MTL only once. If either condition is not met this second time, the Buffer MTL shall not be accepted for any use in the Section and the DNA Technical Leader and manufacturer shall be notified

immediately by the QCO.

5.3.5.4.1.4 See **5.3.1** for naming convention and FA entry.

5.3.5.5 TE Buffer

5.3.5.5.1 QC Testing:

5.3.5.5.1.1 QC testing of TE buffer purchased directly from the manufacturer shall be QC tested as part of QC testing for new lots of Buffer ATL/diluted Buffer ATL.

5.3.5.5.1.2 A sample of the NIST-TS and Neg K shall be extracted, quantitated, amplified, electrophoresed, and analyzed according to applicable Forensic Biology Section DNA Procedures.

5.3.5.5.1.3 The new lot of TE Buffer shall be used to prepare diluted Buffer ATL and shall be used at all steps which require the addition or use of TE buffer (after extraction). An additional Neg K containing only the TE buffer shall be added at the quantitation step.

5.3.5.5.1.4 The expected results for the NIST-TS shall be obtainedfor all loci and the alleles shall be balanced within andbetween loci and peak heights above the analytical threshold and < 15000 RFUs. The Neg K shall be freeof any alleles. If either condition is not met (for reasonsother than instrument failure or known artifacts), then the QCO may retest the new lot of TE buffer only once.If either condition is not met this second time, the TE Buffer shall not be accepted for any use in the Section and the DNA Technical Leader and manufacturer shall be notified immediately by the QCO.

5.3.5.5.1.5 See **5.3.1** for naming convention and FA entry.

5.3.5.5.2 TE Buffer shall be stored at room temperature.

5.3.5.6 Carrier RNA for EZ1 DNA Investigator Kit

Chemical/Reagent	**Amount**
Carrier RNA	**310 µg (one tube)**
Sterile nuclease free dH2O	**310 µL**

5.3.5.6.1 Add water to RNA to reconstitute and mix well.

5.3.5.6.2 Aliquot 50 µL into sterile colored 0.5 mL tubes while under a Biological Safety Cabinet (or equivalent).

5.3.5.6.3 Freeze aliquots immediately at -10 °C. Once aliquot is thawed it shall

not be refrozen and after use. The remainder of the aliquot shall be discarded. The master supply of aliquots shall be stored at -20 °C; working stock supplies of RNA shall be kept at -10 °C. Aliquots expire 1 year after date of preparation, or when stock supply expires, whichever occurs first.

5.3.5.6.4 See **5.3.1** for naming convention and FA entry.

5.3.5.7 Carrier RNA for QIAsymphony DNA Investigator Kit

Chemical/Reagent	Amount
Carrier RNA	**310 µg (one tube)**
Buffer ATE	**1.6 mL**

5.3.5.7.1 Add Buffer ATE (provided in the QIAsymphony DNA Investigator Kit) to RNA to reconstitute and mix well.

5.3.5.7.2 Once added to the reagent cartridge the carrier RNA will be stored at room temperature along with the cartridge to reduce volume loss through condensation on the lid.

5.3.5.7.3 Working stock supplies of dissolved carrier RNA shall be stored at 2-8 °C and expire after 4 weeks after date of preparation.

5.3.5.7.4 For longer storage, dissolved carrier RNA shall be stored at -20 °C. When frozen, dissolved carrier RNA expires 1 year after date of preparation, or when stock supply expires, whichever occurs first.

5.3.5.7.5 See 5.3.1 for naming convention and FA entry.

5.3.5.8 1-Thioglycerol (Casework Direct Kit)

Chemical/Reagent	Amount
1-Thioglycerol	**70 µL**
Water, Amplification Grade	**630 µL**

5.3.5.8.1 Add Water, Amplification Grade to 1-Thioglycerol. Vortex tube for 10-15 seconds.

5.3.5.8.2 Diluted 1-Thioglycerol shall be stored at 4 °C and expires 6 months after date of preparation.

Note: 1-Thioglycerol is viscous. To facilitate accurate pipetting, warm to room temperature, pipette slowly and avoid pipetting small volumes.

5.3.5.8.3 See **5.3.1** for naming convention and FA entry.

5.3.5.9 DNA Quantitation Standards

5.3.5.9.1 The Forensic Scientist shall prepare Standard "1" as described below using the Quantifiler® Trio THP DNA Dilution Buffer and the Quantifiler® THP DNA standard provided in the Quantifiler® Trio Kits. The remaining standards shall be prepared for manual quantification only as a serial dilution starting with Standard 1. Volumes may be adjusted by the Forensic Scientist as long as the dilution factor remains constant.

5.3.5.9.2 Each standard shall be mixed thoroughly and centrifuged before proceeding to the next standard.

5.3.5.9.3 The standards shall be prepared every 2 weeks, or as needed until their expiration date. The standards shall be prepared in sterile 1.5 mLclear plastic tubes.

5.3.5.9.4 Standard "5" will have a higher volume when preparation is complete.

5.3.5.9.5 Quantitation standards shall be stored at 4 °C.

Standard	Amount of Quantifiler Trio THP DNA Dilution Buffer (in µL)	Amount of Standard (in µL)
1	10	10 (stock)
2	90	10 of Standard 1
3	90	10 of Standard 2
4	90	10 of Standard 3
5	90	10 of Standard 4

5.4 QC of Commercial Kits

5.4.1 PowerPlex® Fusion 6C and PowerPlex® Y23: The performance of each lot of Fusion 6C and Y23 shall be checked by the QCO against the NIST-TS as described below prior to use in the Forensic Biology Section.

5.4.1.1 The following items shall be, amplified, electrophoresed and analyzed according to applicable Forensic Biology Section DNA Procedures:

5.4.1.1.1 Standard Traceable to NIST and associated Neg K (previously extracted, if available).

5.4.1.1.2 2800M (positive amplification control).

5.4.1.1.3 Negative Amplification Control.

5.4.1.2 Both the Standard Traceable to NIST and 2800M shall produce the expected results at all loci tested. Alleles shall be balanced within and between loci and peak heights above the analytical threshold and < 15000 RFUs.

5.4.1.3 The Neg K and negative amplification control shall not exhibit any alleles. If multiple Neg K samples are used, at a minimum one must be free of alleles. If a single peak is seen in the second, this does not necessarily need to be repeated. The data will be evaluated by the QCO and DNA Technical Leader and a decision on how to proceed will be documented.

5.4.1.4 The allelic ladder associated with each new lot of Fusion 6C and Y23 shall produce the correct expected alleles.

5.4.1.5 If the kit fails to meet either **5.4.1.2**, **5.4.1.3**, or **5.4.1.4** (for reasons other than instrument failure, known artifacts), it may be retested once. If the kit fails this second re-test, it shall not be accepted for any use in the Section and the DNA Technical Leader and kit manufacturer shall be notified immediately by the QCO.

5.4.1.6 The kit information (lot numbers, date verified, and expiration date) shall be entered into the FA system per **5.3.1.** by the QCO.

5.4.1.7 The general supply of Fusion 6C and Y23 kits shall be stored at -20 °C by the QCO; active working stock shall be kept at 4 °C.

5.4.2 **Quantifiler® Trio:** the performance of each lot of Quantifiler® Trio shall be evaluated by the QCO as described below. The same AB QS5 shall be used throughout the evaluation. The QCO shall use the pipettes designated for QC purposes only.

5.4.2.1 **Kit QC Testing:** Prepare Standard 1 for Qiagility setup or manual setup (per Procedure for DNA Quantitation withQuantifiler Trio) Prepare Master mix according to Biology Workbook using reagents (reaction mix and primer) from the new lot number. QC plate must contain the following: three standard curves (each standard in duplicate), one NTC, one Standard Traceable to NIST and the associated negative extraction control(s) for the Standard Traceable to NIST (if applicable).

5.4.2.2 The DNA Calibrator will also be prepared and tested during the QC of a new lot of Quant Trio.

5.4.2.2.1 The calibrator will either be STD A (DNA Quant Standard) or a previously extracted male DNA sample.

5.4.2.2.2 Quant the calibrator in triplicate to get an average DNA quant for the sample. It is acceptable to prepare dilutions of STD A to get an accurate quant value,

5.4.2.2.3 Using the average quant value, prepare the 5 ng calibrator for casework use.

5.4.2.2.4 If additional tubes of calibrator need to be prepared, use the average value previously obtained.

5.4.2.3 The negative control(s) shall be free of DNA and have an IPC of “Undetermined”. AllStandards Traceable to NIST shall indicate the presence of DNA.

5.4.2.4 Each standard curve shall have acceptable quality metrics as follows:

Target	Slope Range
Small Autosomal (SA)	-3.0 to -3.6
Large Autosomal (LA)	-3.1 to -3.7
Y Target (Y)	-3.0 to -3.6

The R^2 shall be ≥ 0.99.

The three standard curves shall be analyzed together and the average curve metrics entered as the virtual curve for the new quant kit lot number. The virtual curve will be saved as the virtual standard curve and may be set per instrument or per lot number for use in casework.

5.4.2.5 If the quality metrics are not met, the test may be repeated upon the authority and direction of the DNA Technical Leader. If the criteria are successfully met, the new lot of Quantifiler Trio shall be passed for QC.

5.4.2.6 The kit information (lot numbers, date verified, and expiration date) shall be entered into the FA system per **5.3.1** by the QCO.

5.4.2.7 The general supply of Quantifiler® Trio kits shall be stored at -20 °C by the QCO; active working stock shall be kept at 4 °C or -10 °C.

5.4.3 DNA Investigator: Prior to use in the Forensic Biology Section, the performance of each lot of DNA Investigator kits shall be checked by the QCO against the NIST-TS as described below using an amplification kit that is in use within the section.

5.4.3.1 The following items shall be extracted, quantitated, amplified, electrophoresed and analyzed according to applicable Forensic Biology Section DNA Procedures:

5.4.3.1.1 Standard Traceable to NIST.

5.4.3.1.2 Negative Extraction Control (Neg K).

5.4.3.2 The Standard Traceable to NIST shall produce the expected results at all loci tested. Alleles shall be balanced within and between loci and peak heights above the analytical threshold and < 15000 RFUs.

5.4.3.3 The Neg K shall not exhibit any alleles. If multiple Neg K samples are used, at a minimum one must be free of alleles. If a single peak is seen in the second, this does not necessarily need to be repeated. The data will be evaluated by the QCO and DNA Technical Leader and a decision on how to proceed will be documented.

5.4.3.4 If the kit fails to meet either **5.4.3.2** or **5.4.3.3** (for reasons other than instrument failure, known artifacts), it may be retested once. If the kit fails this second re-test, it shall not be accepted for any use in the Section and the DNA Technical Leader and kit manufacturer shall be notified immediately by the QCO.

5.4.3.5 The kit information (lot numbers, date verified, and expiration date) shall be entered into the FA system by the QCO as provided in **5.3.1**.

5.4.3.6 The general supply of DNA Investigator kits shall be stored at 15-25 °C by theQCO.

5.4.4 Qiagen Proteinase K

5.4.4.1 Prior to use in the Forensic Biology Section, the performance check of Qiagen ProK shall be checked by the QCO against the NIST-TS as described below:

5.4.4.1.1 The following shall be extracted, quantitated, amplified, electrophoresed, and analyzed using currently quality control tested kits according to applicable Section procedures: Standard Traceable to NIST and a Negative Extraction Control (NegK).

5.4.4.1.2 The Standard Traceable to NIST shall produce the expected results at all loci tested.

5.4.4.1.3 Alleles shall be balanced within and between loci and give peak heights between approximately 1000 and 15000 RFUs.

5.4.4.1.4 If the Qiagen ProK lot fails to meet either **5.4.4.1.2** or **5.4.4.1.3** (for reasons other than instrument failure or known artifacts), the lot may be retested once. If the Qiagen ProK lot fails this retest, it shall not be accepted for any use in the Section and the DNA Technical Leader and the manufacturer shall be notified immediately by the QCO.

5.4.4.2 The kit information (lot numbers, date verified, and expiration date) shall be entered into FA by the QCO as provided in **5.3.1**.

5.4.4.3 After the lot passes the performance check, aliquot 250 µl into colored sterile 0.5 mLtubes while under a Biological Safety Cabinet (or equivalent).

5.4.4.4 Aliquots shall be stored at 15-25 °C.

5.4.4.5 Aliquots expire 1 year after date of receipt of stock supply.

5.4.5 Casework Direct System: Prior to use in the Forensic Biology Section, the performance of each lot of Casework Direct kits shall be checked by the QCO against a NIST-TS as described below using an amplification kit that is in use within the section.

5.4.5.1 The following items shall be extracted, quantitated, amplified, electrophoresed and analyzed according to applicable Forensic Biology Section DNA procedures.

5.4.5.1.1 Standard Traceable to NIST.

5.4.5.1.2 Negative Extraction Control (Neg K).

5.4.5.2 The Standard Traceable to NIST shall produce the expected results at all loci tested. Alleles shall be balanced within and between loci and peak heights above the analytical threshold and <20000 RFUs.

5.4.5.3 The Neg K shall not exhibit any alleles. If multiple Neg K samples are used, at a minimum one must be free of alleles. If a single peak is seen in the second, this does

not necessarily need to be repeated. The data will be evaluated by the QCO and DNA Technical Leader and a decision on how to proceed will be documented.

5.4.5.4 If the kit failed to meet either **5.4.5.2** or **5.4.5.3** (for reasons other than instrument failure, known artifacts), it may be retested once. If the kit fails this second re-test, it shall not be accepted for any use in the Section and the DNA Technical Leader and kit manufacturer shall be notified immediately by the QCO.

5.4.5.5 The kit information (lot number, date verified, and expiration date) shall be entered into the FA system by the QCO and provided in **5.3.1**.

5.4.5.6 The general supply of Casework Direct kits shall be stored at 2-8 °C by the QCO.

5.4.6 **Bone DNA Extraction Kit**: Prior to use in the Forensic Biology Section, the performance of each lot of Casework Direct kits shall be checked by the QCO against a NIST-TS as described below using an amplification kit that is in use within the section.

5.4.6.1 The following items shall be extracted, quantitated, amplified, electrophoresed and analyzed according to applicable Forensic Biology Section DNA procedures.

5.4.6.1.1 Standard Traceable to NIST or known DNA standard from a previously typed individual

5.4.6.1.2 Negative Extraction Control (Neg K).

5.4.6.2 The Standard Traceable to NIST shall produce the expected results at all loci tested. Alleles shall be balanced within and between loci and peak heights above the analytical threshold and <20000 RFUs.

5.4.6.3 The Neg K shall not exhibit any alleles. If multiple Neg K samples are used, at a minimum one must be free of alleles. If a single peak is seen in the second, this does not necessarily need to be repeated. The data will be evaluated by the QCO and DNA Technical Leader and a decision on how to proceed will be documented.

5.4.6.4 If the kit failed to meet either **5.4.6.2** or **5.4.6.3** (for reasons other than instrument failure, known artifacts), it may be retested once. If the kit fails this second re-test, it shall not be accepted for any use in the Section and the DNA Technical Leader and kit manufacturer shall be notified immediately by the QCO.

5.4.6.5 The kit information (lot number, date verified, and expiration date) shall be entered into the FA system by the QCO as provided in **5.3.1.**

5.4.6.6 The general supply of Bone DNA Extraction Kits shall be stored at 15-25 °C by the QCO. The 1-Thioglycerol shall be stored at 2-8 °C by the QCO.

5.5 Expiration Dates for Commercial Reagents without Manufacturer-Provided Dates

5.5.1 The following reagents shall have an expiration date set 3 years from date of receipt or preparation within the Forensic Biology Section:

- Dithiothreitol (stock supply).
- Top Elute

5.5.2 The following reagents shall have an expiration date set 2 years from date of receipt or preparation within the Forensic Biology Section:

- Hi-Di Formamide (stock supply).
- WEN sizing standard.
- Buffer ATL
- Buffer MTL
- TE Buffer (purchased from an outside supplier)

5.5.3 The following reagents shall have an expiration date set 1 year from date of receipt or preparation within the Forensic Biology Section (unless manufacturer has provided an expiration date):

- Proteinase K provided by Qiagen (aliquots).
- Proteinase K (provided in the DNA Investigator kit).
- Dithiothreitol (aliquots).
- Hi-Di Formamide (aliquots).

5.5.4 For those reagents which are aliquoted, both the date of preparation and expiration shall be marked on the container along with reagent description, initials of preparer, and lot number (unless already covered by previously listed items).

5.5.5 If the reagent container is too small for individual notation of expiration dates, it shall be noted on the parent container (box, bag, bottle or equivalent) storing the main supply of reagents. Lot numbers for reagents can also be checked against FA.

5.5.6 Reagent expiration dates shall be noted in FA by the QCO. Expired reagents shall be disposed of appropriately and not retained in the section

6.0 Limitations - See **5.0**.

7.0 Safety

7.1 DTT: when using this chemical in powder form, masks shall be worn due to the potential as strong respiratory irritants.

7.2 Buffer/Reagent preparation: safety glasses shall be worn at all times when preparing the STR buffers and associated reagents/solutions, unless working behind a BioSafety Cabinet/Fume hood.

7.3 Formamide is a known chemical hazard and can cause eye, skin and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Wear appropriate eyewear, gloves and clothing when in use.

7.4 Assume that all body fluids contain bloodborne pathogens and handle accordingly.

7.5 If the examination involves a biohazard, wear proper personal protective equipment such as eye protection, lab coat, and / or gloves.

8.0 References

Forensic Biology Section Procedure for Safety and Hazardous Waste Disposal
Forensic Biology Section Procedure for the Casework Direct System
Forensic Biology Section Procedure for DNA Extraction
Forensic Biology Section Procedure for DNA Extraction of Bone and Teeth
Forensic Biology Section Procedure for PCR Amplification for Casework
Forensic Biology Section Procedure for Human DNA Quantitation with Quantifiler® Trio
Forensic Biology Section Procedure for Use of the 3500 Genetic Analyzer for Casework
Forensic Biology Section Procedure for Body Fluid Unit Quality Control
Forensic Biology Section Procedure for Aseptic Technique and Contamination Control

9.0 Records

- Temperature Charts for Freezers/Refrigerators
- QC Testing Worksheets
- PowerPlex® Fusion 6C QC Form
- PowerPlex® Y23 QC Form
- Quantifiler® Trio Kit QC Form
- QC Testing Worksheet Templates
- DNA Investigator Kit QC Form
- Casework Direct System QC Form
- Bone DNA Extraction Kit QC Form

10.0 Attachments

- Appendix I – Chemical Hygiene and Safety Precautions for Particularly Hazardous Substances

Revision History		
Effective Date	Version Number	Reason
05/24/2024	13	5.3.5.11.2 – clarify storage of QIAsymphony components; Updated numbering throughout

Appendix I – Chemical Hygiene and Safety Precautions for Particularly Hazardous Substances

Hi-Di Formamide **DANGER: PARICULATLY HAZARDOUS SUBSTANCE** *	
	**HEALTH 2** **FLAMMABILITY 1** **REACTIVITY 0**
Detection of Release	Clear odorless liquid.
Signs/Symptoms of Exposure	May cause skin and eye irritation
PEL	NIOSH Recommended Exposure Limits (TWA) 10 ppm USA
Associated Hazards	Suspected of causing cancer. May damage fertility or the unborn child. Danger of cutaneous absorption. May cause damage to organs (Blood) through prolonged or repeated exposure if swallowed. May cause liver and kidney damage.
Controls	Use under fume hood. Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product. Use tight sealing safety goggles. Handle with gloves. Wear lab coat. Gloves: nitrile (break through time > 1 hour).
Safe handling, storage, disposal	Avoid contact with skin and eyes. Avoid inhalation of vapor or mist. Keep in a tightly closed container. Store in a cool, dry, corrosion-proof, ventilated area away from moisture, sources of heat or ignition, combustibles and oxidizers. Protect against physical damage. Dispose of in Hazardous Chemical Waste.
Emergency Procedures	**Eye Contact:** Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Immediate medical attention is required. **Inhalation Exposure:** Remove to fresh air. If not breathing, give artificial respiration. If symptoms persist, call a physician. **Ingestion:** Never give anything by mouth to an unconscious person. Do not induce vomiting without medical advice. If swallowed, rinse mouth with water (only if the person is conscious). Risk of serious damage to the lungs (by aspiration). Get medical attention if symptoms occur. **Skin Contact:** Wash off immediately with plenty of water for at least 15 minutes. Remove and wash contaminated clothing and gloves, including the inside, before re-use. Immediate medical attention is required. **Spills:** Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Small contained spill: wearing appropriate PPE, soak up with inert absorbent material, and place in container. Dispose in Hazardous Waste. Large spills: Evacuate area and call 911 (Haz Mat).