Procedure for Calibration and Equipment Maintenance

1.0 Purpose - This procedure specifies the required elements for the performance check, verification and maintenance of equipment used by the Forensic Biology Section as performed by the DNA Quality Control Officer or designee(s).

2.0 Scope – This procedure applies to equipment used by the Forensic Biology Section.

3.0 Definitions – See section Definition list

4.0 Equipment, Materials and Reagents

- NIST traceable digital thermometer
- Ice Shaver/crusher
- Purified dH_2O
- dH_2O
- NIST traceable weight set
- Spectral calibration kits (for ABI Quant Studio 5 (QS5), 3500 or equivalent)
- Microwave
- ~1000 mL beaker
- Syringe
- Septa
- diluted Buffer ATL
- Wipes (delicate task wipes)
- 50 mL conical tube
- 96 well reaction trays
- Pipettes
- Pipette tips
- Matrix standard set DG4900 and DG4850 to analyze automatically the six and five different colored fluorescent dye-labeled samples in a single capillary and PowerPlex™ 6C and Y23 Matrix Standards and Matrix Dilution Buffer.
- NIST-TS
- ABI 3500 anode and cathode buffers
- Formamide/WEN master mix2800M control DNA
- Mineral oil

5.0 Procedure – All documentation for post-maintenance or repair shall be reviewed by the Technical Leader prior to the instrument being returned to service. The exceptions to this requirement are the monthly QS5 maintenance as it does not require the instrument to be taken off-line to complete and pipettors and probes that are calibrated by an external vendor. For equipment that undergoes external calibrations (e.g., data loggers, pipettors, digital probes) the performance check will consist of a review of the calibration documentation provided by the vendor.

5.1 AB QS5 Real-Time PCR System Instrument Maintenance

5.1.1 The following table displays the recommended QS5 instrument and laptop maintenance schedule ensuring proper operation of the instrument (found in the AB Quant Studio Real-Time PCRSystems System Maintenance Guide (Publication # MAN0017162 Rev A.0, 6/15/2017). Monthly, quarterly,

and annual maintenance tasks should be performed using the listed steps/reference information at the frequencies indicated by the instrument manual. All tasks except for annual maintenance shall be performed under the direction of the QCO and all records shall be maintained in the section. Monthly maintenance tasks are already included during annual maintenance.

Frequency	Maintenance Task*
Weekly	Check disk space and reboot computer
	Power off the instrument for at least 30 seconds
	Wipe surface of instrument and computer with lint free cloth
Monthly	Decontaminate the sample block
	Perform instrument self-test
	Run background calibration
	Remove data files from instrument (Not computer)
Annually	Performed by AB engineer

Calibration	Recommended frequency
ROI/Uniformity	• Every two years (recommended) • Always perform new background and dye calibrations after an ROI/uniformity calibration. **Note:** Performing an ROI/uniformity calibration invalidates all other calibrations.
Background	• Every two years (recommended) • Background calibration can also be performed, as needed: – To check for contamination (depends on usage and laboratory conditions). – To obtain the most accurate data for the removal of background fluorescence. **Note:** Performing a background calibration does *not* invalidate any other calibration.
Dye (*system and custom dyes, including ABY™ and JUN™ dyes*)	• Every two years (recommended) • During a dye calibration, only the dyes on the given spectral calibration plate are calibrated. **Note:** Performing a dye calibration for a given dye plate does *not* invalidate any other calibration.
RNase P instrument verification	• After performing instrument calibrations • As needed to confirm instrument performance

As needed	Decontaminate block
	Computer hard drive-run disc clean-up defragmentation
	Update windows operating system or QS5 software- Service Call
	RNASE P verification (NOTE: RNase P verification is typically only performed at the request of AB Technical Support for troubleshooting purposes)

5.1.2 Repair: If an ABI QS5 becomes inoperable due to a need for repair by the manufacturer, the QCO shall immediately notify the DNA Technical Leader and manufacturer. Additionally, the QCO shall notify all members of the Forensic Biology Section via email and place a notice on the specific instrument that it is not available for use.

5.1.3 Post Annual Maintenance/Repair Performance QC Check: Before any validated AB QS5 may be used for analysis following repair or annual preventive maintenance, a QC check shall be performed by the QCO. This QC check shall be performed as follows:

5.1.3.1 An RNaseP plate may be run or a NIST-TS (see DNA Reagent Preparation and Quality Control Procedure) shall be quantitated (i.e., Quantifiler Trio) with the DNA Standard Curve in duplicate (10 total data points), a calibrator, and one Negative Template Control (NTC).

5.1.3.2 Items listed above shall be quantitated in accordance with the Procedure for DNA Quantitation.

5.1.3.3 The NIST-TS shall indicate the presence of DNA. All testing negatives shall have an IPC C_t value of ≥ 40 or a value stating it is "Undetermined."

5.1.3.4 Quality metrics of the standard curves (Small autosomal, Large Autosomal, and TY) shall fall within acceptable QC ranges.

5.1.3.5 If either **5.1.3.3** or **5.1.3.4** is not satisfied, the QCO shall repeat the QC check.

5.1.3.6 The QCO shall notify the Section via email as well as by placing a notice on the specific instrument that it is again available for use once the QC check is completed.

5.1.3.7 The QCO shall document the testing performed and retain them in the appropriate QC files with the specific AB QS5 maintenance records until it is scanned into the Section shared folder.

5.2 ABI 3500 Genetic Analyzer

5.2.1 Weekly/Monthly Maintenance shall be performed as per the "Applied Biosystems 3500/3500xL Genetic Analyzer User Guide; Part Number 4401661; Revision C and shall be performed at a minimum of every two weeks.

5.2.1.1 Weekly/Bi-weekly Tasks:

5.2.1.1.1 Restart the computer and instrument.

5.2.1.1.2 Check storage conditions of the used arrays to ensure the array tip is covered in the reservoir.

5.2.1.1.3 Run the Wash Pump and Channels wizard, finish with polymer pump flush and capillary array fill. Run bubble wizard as necessary.

5.2.1.1.4 Use the lab wipe to clean the anode buffer container valve pin assembly on the polymer delivery pump.

5.2.1.1.5 Change the anode and cathode buffers and replace cathode buffer septa.

5.2.1.1.6 To flush capillaries, run a WEN plate– Prepare master mix for an entire injection (e.g., 8 or 24 depending on instrument). Dispense 10 µl of formamide/WEN master mix for one full injection and inject using standard conditions.

5.2.1.2 Monthly Tasks:

5.2.1.2.1 Using a syringe of deionized water (~15 ml), flush the pump trap.

5.2.1.2.2 Using a lab wipe, clean the autosampler and drip tray.

5.2.1.2.3 Perform computer maintenance.

5.2.1.2.4 Monitor the number of plate records in the software. Delete/archive as necessary. Note: Storing >500 plate records on the instrument may cause errors in plate linking.

5.2.2 Spatial Calibration

5.2.2.1 Spatial calibration shall be performed as per the "Applied Biosystems 3500/3500xL Genetic Analyzer User Guide; Part Number 4401661; Revision C.

5.2.2.2 Spatial Calibration shall be performed when:

5.2.2.2.1 The capillary array has been removed or replaced.

5.2.2.2.2 The detector door has been opened or the detection cell has been moved.

5.2.2.2.3 The instrument has been moved.

5.2.3 Spectral Calibration

5.2.3.1 Spectral calibration shall be performed as per the "Applied Biosystems 3500/3500xL Genetic Analyzer User Guide; Part Number 4401661; Revision C.

5.2.3.2 Spectral Calibration shall be performed when:

5.2.3.2.1 A service engineer has performed an optical service procedure such as realigning or replacing the laser or CCD camera or mirrors on the instrument.

5.2.3.2.2 A decrease in spectral separation (pull-up) in the raw or analyzed data has been observed.

5.2.3.3 Changing the Capillary Array - When a capillary has repeated ILS (i.e., sizing standard) failure, or the bases of the alleles in samples broaden, or the background noise in the electropherograms becomes repeated and excessive (based upon the training and experience of the Forensic Scientist), the array shall be replaced. Forensic Scientists shall notify the QCO and DNA Technical Leader if they observe any of the above-mentioned scenarios.

5.2.3.3.1 Changing the array shall be performed as per the "Applied Biosystems 3500/3500xL Genetic Analyzer User Guide; Part Number 4401661; Revision C."

5.2.3.3.2 Once completed, a spatial calibration shall be performed. A spectral calibration may be performed.

5.2.4 Service and/or Repair

5.2.4.1 Repair: If a 3500 becomes inoperable, the QCO shall notify the Section via email as well as by placing a notice on the specific instrument that is not available for use. The QCO

shall also notify the DNA Technical Leader and the manufacturer that repair is needed.

5.2.4.2 Performance QC Check: If a 3500 instrument is removed from use due to repair, a post maintenance QC check on the instrument shall be performed by the QCO prior to its return to use in the Section.

5.2.4.3 Laser Failure/Replacement

5.2.4.3.1 If the Laser is replaced, a spatial and spectral calibration shall be performed.

5.2.4.3.2 The QCO shall then perform a Post Maintenance Performance QC Check on the instrument.

5.2.4.3.3 Additionally, a sensitivity study shall be performed on the instrument by the QCO at the direction of the DNA Technical Leader.

5.2.4.3.4 After all conditions are satisfied, the DNA Technical Leader shall release the instrument for use in Casework. The QCO shall notify the Section by email and by placing a notice on the specific instrument that it is available for use.

5.2.5 Annual Preventative Maintenance

5.2.5.1 The ABI 3500 Genetic Analyzers shall have preventative maintenance performed annually by the manufacturer for instruments in use by the section for the entire year.

5.2.5.2 Performance QC Check: After preventative maintenance, each 3500 shall have a post maintenance QC check performed by the QCO.

5.2.6 Documentation of any repair or annual preventative maintenance, as well as subsequent QC Checks, shall be retained in the Section indefinitely.

5.2.7 **Post Maintenance Performance QC Check:** Before any validated 3500 shall be used by Forensic Scientists in the Forensic Biology Section after repair or maintenance, a Performance QC check shall be performed by the QCO as described below. When a 3500 instrument is either removed or returned to service, the QCO shall notify the Section via email and place a notice on the instrument regarding its status. All QC documentation shall be retained in the appropriate QC files with the specific 3500 maintenance records. DNA TL approval shall be obtained prior to return to service of any 3500.

5.2.7.1 **Post Annual Preventative Maintenance:** If no modifications to the optical components of a 3500 are made during annual preventative maintenance (PM), the following instrument assessments shall be performed:

5.2.7.1.1 Precision: A master mix of ladder/formamide/WEN sizing standard shall be prepared to fill a complete injection (e.g., 24 wells or 3 columns) on a single plate and injected at normalized conditions. The ladder shall be analyzed for precision such that all alleles within the allelic ladder have standard deviations below 0.15 bp.

5.2.7.1.2 Traceability: After precision (and normalization, if required) has been successfully achieved, at least one previously typed standard must be amplified

with the appropriate amplification positive and negative control(s) and injected on the serviced instrument. The standard, positive amplification control and allelic ladder must provide the expected allele calls at all loci tested. All negative controls must be free of alleles.

5.2.7.1.3 Normalization: Several samples of at least 2 previously typed standards from different sources shall be amplified and PCR product pooled (for each) to fill one complete injection (24 wells or 3 columns, including one well set aside for allelic ladder). Inject one set at normalized conditions (injection time used prior to service) to establish normalization using the most current data from other casework dedicated 3500XL instruments. Repeat injection times on serviced instrument with different injection times as necessary until criterion is satisfied (peak heights should be within ~10 % between instruments).

5.2.7.1.4 Note: If any of the above assessments are not satisfied, the QCO may repeat the assessment one additional time. If the assessment fails a second time, **proceed to 5.2.7.2** or place a service call to the manufacturer as necessary.

5.2.7.2 **After Repair/Post PM QC Failure:** After any repair to the optics system of a 3500 (e.g., laser replacement), or if post annual maintenance QC fails, the following assessments shall be performed:

5.2.7.2.1 Precision and traceability (see **5.2.7.1.1** and **5.2.7.1.2**).

5.2.7.2.2 Sensitivity: A dilution series of a previously typed known reference shall be prepared beginning with a total initial input target of 2 ng and serially diluted to 0.031 ng, with an additional 0.100 ng dilution prepared. The serial dilutions shall be quantified in triplicate and their concentrations adjusted accordingly (if necessary). The dilutions shall then be amplified in triplicate and injected on the 3500 at previously established conditions. All data within 60 to 500 bp shall be assessed for minimum threshold (2[max-min], Limit of Detection, Limit of Quantification), peak height imbalance (peak height ratios), and stochastic thresholds (allelic drop-out). Thresholds and PHR shall fall within existing specifications. Note: Other well-characterized DNA samples may be used as appropriate for this assessment.

5.2.8 Documentation of any repair or annual preventative maintenance, as well as subsequent QC Checks, shall be retained in the Section indefinitely.

5.3 AB ProFlex Thermal Cyclers

5.3.1 Internal Verification: All thermal cyclers currently in service within the Section shall be subjected to a series of temperature verifications on an annual basis by the QCO. Caution shall be exercised at all times as the thermal cyclers can reach temperatures in excess of 100 °C. The results for these tests are exportable as a .log files which can be opened in Excel and then printed to PDF. This documentation shall be retained indefinitely by the QCO.

5.3.1.1 Temperature Non-Uniformity: A set of six zones on each thermal cycler shall be tested for two

temperature groups: 95 °C and 60 °C. For each temperature group, the range between the highestand lowest values shall not exceed +/- 0.5 °C from the set temperature for the test to pass.

5.3.1.1.1 Turn on the thermal cycler, from the home screen touch "Settings," "Maintenance & Services," "Block Verification Test," "Verify Block Temperature," and "Temperature Non-Uniformity."

5.3.1.1.2 Touch "Next." Connect the Temperature Verification Kit (TVK) to instrument. Follow the instructions on the screen for placement of the TVK probe in the block.

5.3.1.1.3 Touch "Start Test." Follow the instructions on the screen.

5.3.1.1.4 Once the test has finished, the instrument display will indicate "passed" or "failed" for each of the zones tested.

5.3.1.1.5 Insert a USB into the USB drive, touch "Export" to export the Temperature Non-Uniformity log.

5.3.1.2 Temperature Verification: A set of six zones on each thermal cycler shall be tested for two temperature groups: 85°C and 45°C . For each temperature group, the range between the highest and lowest values shall not exceed +/- 0.25 °C of the set temperature to pass.

5.3.1.2.1 Turn on the thermal cycler, from the home screen touch "Settings," "Maintenance & Services," "Block Verification Test," "Verify Block Temperature," and "Temperature Verification."

5.3.1.2.2 Touch "Next." Connect the Temperature Verification Kit (TVK) to instrument. Follow the instructions on the screen for placement of the TVK probe in the block.

5.3.1.2.3 Touch "Start Test." Follow the instructions on the screen.

5.3.1.2.4 Once the test has finished, the instrument display will indicate "passed" or "failed" for each of the zones tested.

5.3.1.2.5 Insert a USB into the USB drive, touch "Export" to export the Temperature Verification log.

5.3.1.3 Heated Cover Test: The Temperature Verification Kit (TVK) is used in central zones 3 and 4 to verify that the heat cover is producing temperatures within +/- 3°C of the target temperature of 105°C.

5.3.1.3.1 Turn on the thermal cycler, from the home screen touch "Settings," "Maintenance & Services," "Block Verification Test," "Verify Block Temperature," and "Heated Cover."

5.3.1.3.2 Touch "Next." Connect the Temperature Verification Kit (TVK) to instrument. Follow the instructions on the screen for placement of the TVK probe in the block.

5.3.1.3.3 Touch "Start Test." Follow the instructions on the screen.

5.3.1.3.4 Once the test has finished, the instrument display will indicate "passed" or "failed" for the test.

5.3.1.3.5 Insert a USB into the USB drive, touch "Export" to export the Heated Cover Test log.

5.3.1.4 Self- Verification Test: The Self-Verification test feature can be used to check the instrument hardware. This test can be performed if there is an intermittent error. The check includes testing the block, heated cover, and other components.

5.3.1.4.1 In the Home screen, touch "Settings," "Maintenance & Services," and "Self Verification Test."

5.3.1.4.2 Touch "Start Test" to begin testing.

5.3.1.4.3 Once the test has completed, the test results will be displayed in the form of a report and can be exported.

5.3.1.4.4 Insert a USB into the USB drive, touch "Export" to export the Self Verification Test report.

5.3.2 Notification for Use: If any thermal cycler fails any of these tests, the QCO shall immediately notify the DNA Technical Leader, as well as the Section via email and a "Do Not Use" sticker shall be placed on the affected instrument.

5.3.3 Performance QC Check: If a thermal cycler requires a QC check after repair or before a new instrument is put on-line that was not used as a part of the associated validation studies, a QC check shall be performed by the QCO, in addition to the tests as described in **5.3.1**.

5.3.3.1 This QC check shall consist of the amplification of the following:

5.3.3.1.1 Positive amplification control (2800M) and negative amplification control (Neg Amp), using the current amplification kit.

5.3.3.1.2 A previously typed known reference sample.

5.3.3.1.3 Five sets of a previously typed known reference sample shall be amplified at the following well locations and electrophoresed and analyzed per DNA procedures:

5.3.3.2.1 E1-H1, C4-F4, B7-E7, E10-H10, A12-D12.

5.3.3.2 The expected results for the previously typed known reference sample, positive amplification controls, and allelic ladders 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. All negative controls shall be free of any peaks or activity. If any of these conditions are not met (for reasons other than instrument failure or known artifacts), then the QCO may retest the affected wells in the thermal cyclers once. If the conditions are not

met this second time, the QCO shall keep the thermal cycler offline and notify the DNA Technical Leader and manufacturer. If the thermal cycler is under a manufacturer warranty, the manufacturer shall be contacted for repair. If the thermal cycler is no longer under any warranty, the section manager shall be contacted for decision of how to proceed.

5.3.4 External Calibrations/Verification: If the thermal cyclers are verified by an external vendor, the results shall be documented. The thermal cyclers that are passed by the external vendor shall be accepted as calibrated/verified and noted as such until the next annual verification is due. This documentation shall be retained indefinitely by the QCO.

5.4 Digital Probes

5.4.1 Annual External Calibration: the digital probes (Eutechnics 4500 or equivalent) shall be calibrated annually by a contract vendor against an appropriate NIST traceable standard.

5.5 Bulb Thermometers

5.5.1 Purpose/Use: May be used to measure temperatures in incubators. Surplus calibrated bulb thermometers shallbe retained by the QCO, unless broken, and then disposed of in accordance with the Section Safety policy and procedures.

5.5.2 Annual Internal Performance Check: All bulb thermometers in use within the Forensic Biology Section shall be checked on an annual basis internally against a NIST traceable thermometer (i.e., the "NIST lollipop") in an ice bath.

5.5.2.1 Freeze several trays of dH_2O into ice cubes; once frozen, grind or crush them in an ice shaver (or equivalent). Mix the ice shavings with dH_2O and place into an insulated container deep enough (thermos or equivalent) to contain the metal probe portion of the NIST Traceable Thermometer.

5.5.2.2 The QCO shall wipe down each bulb thermometer with fresh 10 % bleach followed by an ethanol rinse and allow it to dry (either through evaporation or wiping with a wipe) before inserting it into the ice bath.

5.5.2.3 Using clamps and foam (or equivalent) to hold both the NIST traceable thermometer and the bulb thermometer to be calibrated within an inch of each other in the ice bath, wait for the NIST traceable thermometer to register 0.0 °C. Be sure to align the bulb thermometer such that the bulb portion is submerged in the ice bath, but that the area marked for 0.0 °C can be visualized by the QCO.

5.5.2.4 Once the NIST traceable thermometer reads 0.0 °C, record the temperature to the nearest tenth of a degree on the bulb thermometer. If the bulb thermometer is greater than +/- 1 °C from the NIST traceable thermometer, it shall be destroyed and replaced with a calibrated bulb thermometer.

5.5.2.5 The QCO shall record both the NIST traceable thermometer and calibrated bulb thermometer readings on the Bulb Thermometer Temperature Performance Check Form. The QCO shall also create and place a sticker on each calibrated bulb thermometer that

indicates the specific bulb thermometer number, the date the next performance check is due, the initials of the QCO performing the check, and whether the user of the bulb thermometer shall add or subtract tenths of a degree to the reading of that bulb thermometer to bring it to specifications as indicated by the NIST traceable thermometer (i.e., if the bulb thermometer reads 0.5 °C higher than the NIST traceable thermometer, the Forensic Scientist shall subtract 0.5 °C from the bulb thermometer reading before recording a temperature).

5.5.3 This process shall be completed for all bulb thermometers, including those set aside for storage or future use (i.e., replacement).

5.5.3.1 Documentation of the performance checks shall be retained indefinitely by the QCO in the Section.

5.6 **Digital Thermometers**: Purchased from external vendor; shall be NIST traceable and replaced when NIST traceability expires. Digital thermometers shall be used to monitor temperatures on incubators, freezers and refrigerators in the Section as needed. Surplus digital thermometers may be retained by the QCO.

5.7 **NIST Traceable Thermometer** (i.e., the "NIST lollipop"): Has an elongated metal probe which is used for testing against bulb thermometers/thermomixers purchased from an external vendor; shall be NIST traceable and replaced when NIST traceability expires.

5.8 **Thermomixer** – Thermomixers are used within the Forensic Biology to ensure samples are at the proper temperatures, used during extraction, and used when reconstituting dried extracts. All thermomixers in use within Forensic Biology shall be performance checked at least annually. Temperature verification will be performed prior to a thermomixer initially being used in casework or before being returned to service following repair or maintenance.

5.8.1 All thermomixers shall be temperature checked against a NIST traceable thermometer (e.g., NIST lollipop or equivalent traceable).

5.8.2 The thermomixer will be turned on and set to the temperature used during casework analysis. Diluted Buffer ATL will be added to six extraction tubes and each tube will be placed in a well. The wells chosen will be random and spread throughout the available wells. The wells chosenmust be recorded. The tubes will be allowed come to the set temperature.

5.8.3 Using a NIST traceable thermometer, the temperature of each of the six wells that contain a tube with buffer shall be recorded along with the set temperature.

5.8.4 The process shall be repeated at all temperature settings used during casework analysis in Forensic Biology (e.g., 56 °C, 70 °C).

5.8.5 If the temperature readings are not within 5 °C of the set reading, the thermomixer must be taken offline and the Technical Leader will be notified. Documentation will be maintained within Forensic Biology.

5.9 **Balances -** External Calibrations: All balances in the Forensic Biology Section shall be calibrated at least annually by a contract vendor. The weight set in the Forensic Biology Section shall be calibrated annually.

5.10 **Pipettors**

5.10.1 Annual External Calibrations: All pipettors in the Forensic Biology Section shall be calibrated at least

annually by a contract vendor.

5.10.2 Repair: If a pipettor breaks or a Forensic Scientist, based on their training and experience believes that the pipettor does not work properly, it shall be given to the QCO for storage until an external calibration vendor can repair and calibrate it. If the pipettor is not repairable, it shall be removed from the Section.

5.11 Biosafety Cabinets/Chemical Fume Hoods/Laminar Flow Clean Air Benches

5.11.1 Annual External Calibrations: All Nuaire Biological Safety Cabinets, Chemical Fume Hoods, and Laminar Flow Clean Air Benches (amplification hoods) in the Section shall be calibrated annually by a contract vendor.

5.11.2 Any hood listed in **5.11.1** that does not pass certification shall not be used.

5.12 Freezers/Refrigerators

5.12.1 Recording Temperatures: The QCO shall record daily temperatures for all common area refrigerators/freezers in the Section used for critical reagents and/or evidence storage; however, if the QCO has not yet recorded the temperatureand a Forensic Scientist uses a common area refrigerator/freezer, the Forensic Scientist shall record the temperatures prior to opening the door(s). Temperatures are recorded on a Temperature Recording Form (TRF).

5.12.2 -20 °C Freezers: These freezers shall not vary more than + 5 °C from the set temperature. The temperature for these freezers shall be recorded by personnel using the TRF as described in **5.12.1**.

5.12.2.1 The QCO shall fill out all required information regarding freezer serial number, the location of the freezer, the set temperature of the freezer, and the associated digital thermometer serial number at the beginning of every calendar year on a TRF for each common area -20 °C freezer.

5.12.2.2 If at any point during the calendar year a new digital thermometer is needed, the QCO shall write at the bottom of the TRF the date on which a new thermometer was used and the serial number for the new thermometer.

5.12.2.3 If a -20 °C freezer must be thawed, the contents of the freezer shall immediately be moved to another -20 °C freezer that is within range and the QCO shall note this, as well as the affected dates, on the TRF. The contents shall not be returned to the original -20 °C until the temperature is within range.

5.12.2.4 If the temperature for a -20 °C freezer exceeds the + 5 °C consistently for more than 5 consecutive business days, QCO shall immediately move the contents to another -20 °C freezer that is within range and note this, as well as the affected dates, on the TRF. The contents shall not be returned to the original -20 °C freezer until the temperature is within range.

5.12.3 -10 °C/4 °C Freezer/Refrigerator Units: These units shall not vary more than + 5 °C from the set

temperature(s) for the freezer portion; the refrigerator portion shall not fall below 2 °C or exceed 8 °C. The temperature for these freezers shall be recorded using the TRF by personnel as described in **5.12.1**.

5.12.3.1 The QCO shall fill out all required information for common area -10 °C/4 °C freezer/refrigerator units regarding the unit serial number, location, set temperatures, and the associated digital thermometer serial number on the TRF. For limited access -10 °C/4 °C freezer/refrigerator units, the Forensic Scientist(s) that have access to such units shall fill out the information on a TRF.

5.12.3.2 If at any point during the calendar year a new digital thermometer is needed, the QCO shall be notified and the new thermometer serial number shall be recorded on the TRF associated with the refrigerator/freezer.

5.12.3.3 If the QCO or Forensic Scientist observes temperatures out of the range specified in **5.12.3** for more than five consecutive business days, then the QCO (for common area units) or the Forensic Scientist (limited access units), shall attempt to adjust the temperature back in range using the thermostat for the unit. If the temperature does not come within range within a 24-hour period, the QCO (or Forensic Scientist) shall transfer the contents of the unit to another unit with the same temperature parameters and note on the TRF the unit to which the contents were transferred and the date of transfer. If additional adjustments of the thermostat are unsuccessful, the unit shall be removed from service and clearly marked as being out of service. If additional adjustments are successful at restoring the unit to the temperatures specified in **5.12.3**, then the contents may be returned to the unit.

5.13 **Incubators**: Temperatures shall be recorded on the day(s) the incubator is in use. If the incubator is in a common area, the QCO shall record the temperature. If the incubator is in a shared suite, the Forensic Scientist shall record the temperature. Temperatures shall be recorded on a TRF specific for the incubator.

5.13.1 The QCO or Forensic Scientist shall fill out all required information regarding the unit serial number, location, set temperatures, and the associated bulb thermometer number on the TRF.

5.13.2 The incubators shall be +/- 5 °C degrees within the set temperature. If an incubator consistently deviates more than this over a period of five consecutive readings, then the QCO or Forensic Scientist shall attempt to adjust the temperature back into the acceptable range over a period of 24 hours. If all attempts at obtaining a set temperature within range fail, the QCO shall be notified and the incubator removed from service and marked as such.

5.14 All verification, calibration, maintenance, and QC documentation shall be retained in the Forensic Biology Section.

5.15 When any of the following instruments/equipment need repair and are taken out of use from the Section, the QCO shall notify the DNA Technical Leader, and if necessary, the manufacturer. The QCO shall also notify the DNA Technical Leader when the instruments/equipment are suitable for use by the Section.

- 3500(XL), AB QS5, ABI 9700, QIAgility, QIAcube, EZ1 Advanced XL BioRobot, EZ2 Connect Fx BioRobot, QIAsymphony BioRobot, Centrifuges, Hoods, Freezers/Refrigerators, Balances.

5.16 **QIAgility**

5.16.1 Annual Preventative Maintenance

5.16.1.1 Annual Preventative Maintenance: the QIAgility shall have preventative maintenance performed annually by the manufacturer.

5.16.1.1.1 Refer to the Planned Maintenance Protocol (record) provided by the manufacturer for specific calibrations, verifications, and tests performed during the annual preventative maintenance.

5.16.1.1.2 Performance QC Check: After preventative maintenance, each QIAgility shall have a post maintenance QC check performed by the QCO.

5.16.2 Weekly Maintenance

5.16.2.1 Wipe the outside of the instrument with a dust cloth or lab wipe dampened with deionized water.

5.16.2.2 Remove all loading blocks and the tip ejector chute from the worktable. Rinse these with ethanol and dry.

5.16.2.3 Wipe the worktable down with ethanol and return the tip ejector chute and the loading blocks to the worktable. Note: The tip ejector may be recalibrated at the direction of the QCO or manufacturer. To recalibrate, click Options in the menu bar, click Setup tip ejector and follow the screen prompts.

5.16.2.4 Close the lid and turn on the UV lamp. Click the light bulb on the icon bar at the top of the screen. Click the box marked "Close software when UV finished?" and set the time to 15 minutes.

5.16.2.5 Click Start and then click "Yes" when the alert screen opens.

5.16.2.6 Restart the computer attached to the Qiagility instrument.

5.16.2.7 Document the maintenance on the Forensic Biology Section QIAgility Maintenance Form (located with the instrument).

5.16.2.7.1 The QCO shall retain such information in the QC files with the specific instrument maintenance records.

5.16.3 Post Maintenance Performance QC Check: After repair or preventative maintenance, the QCO shall review documentation provided by the engineer. The QC check shall consist of reviewing the planned maintenance and calibration records for targeted values to verify that the instrument is working correctly. All testing specifications listed within this documentation shall be marked as "Pass" by the engineer or manufacturer. This documentation will be retained for maintenance records.

5.17 EZ1 Advanced XL and EZ2 Connect Fx BioRobot

5.17.1 Annual Preventative Maintenance

5.17.1.1 Annual Preventative Maintenance: the EZ1Advanced XL and EZ2 Connect Fx BioRobots shall have preventative maintenance performed annually by the manufacturer.

5.17.1.1.1 Refer to the Planned Maintenance Protocol (record) provided by the manufacturer for specific calibrations, verifications, and tests performed during the annual preventative maintenance

5.17.1.1.2 Performance QC Check: After preventative maintenance, each EZ1 and EZ2 shall have a post maintenance QC check performed by the QCO.

5.17.2 Cleaning/Maintenance – See the Section Procedure for DNA Extraction.

5.17.3 Post Maintenance Performance QC Check: Before any validated EZ1 and EZ2 may be used after repair orpreventative maintenance, the QCO shall review documentation provided by the engineer. The QC check shall consist of reviewing the preventative maintenance records. All testing specifications listed within this documentation shall be marked as "Pass" and the operational verification tests approved by the engineer via signature. This documentation will be retained for maintenance records.

5.18 Qiagen QIACube

5.18.1 Annual Preventative Maintenance

5.18.1.1 Annual Preventative Maintenance: the Qiagen QIACube shall have preventative maintenance performed annually by the manufacturer.

5.18.1.1.1 Refer to the Planned Maintenance Protocol (record) provided by the manufacturer for specific calibrations, verifications, and tests performed during the annual preventative maintenance.

5.18.1.1.2 Performance QC Check: After preventative maintenance, each Qiagen QIACube shall have a post maintenance QC check performed by the QCO.

5.18.2 Cleaning

5.18.2.1 Daily Cleaning: After use, each analyst shall wipe the instrument interior with ethanol/alcohol. All reagent bottles and consumables shall be checked and restocked as necessary. Document cleaning on the cleaning/maintenance form.

5.18.2.2 Monthly Cleaning: QCO will follow the prompts on the instrument touchscreen.

5.18.2.2.1 Wipe optical sensor, tip adaptor, gripper unit and stabilization rob with a lint free wipe.

5.18.2.2.2 Clean the rotor, buckets, dH20 bottles and tip collector with soap and water. If build-up is present on rotor or buckets, clean with a toothbrush to remove. Then wipe with ethanol/alcohol.

5.18.2.2.3 Wipe inside of centrifuge with rotor removed with ethanol/alcohol.

5.18.2.2.4 Apply mineral oil to rotor prior to returning it the centrifuge. Also apply to buckets prior to replacement in the instrument.

5.18.2.2.5 Document cleaning on the cleaning/maintenance form.

5.18.2.3 As needed, perform the tightness test as directed by screen prompts/instrument manual.

5.18.3 Post Maintenance Performance QC Check - Before any validated Qiacube may be used after repair or preventative maintenance, the QCO shall review documentation provided by the engineer. The QC check shall consist of reviewing the preventative maintenance records. All testing specifications listed within this documentation shall be marked as "Pass" and the operationalverification tests approved by the engineer via signature. This documentation will be retained for maintenance records.

5.19 QIAsymphony

5.19.1 Annual Preventative Maintenance

5.19.1.1 Annual preventative maintenance (PM): The Qiagen QIAsymphony shall have preventative maintenance performed annually by the manufacturer.

5.19.1.1.1 Refer to the planned maintenance protocol (record) provided by the manufacturer for specific calibrations, verifications, and test performed during the PM.

5.19.1.1.2 Performance QC check: After the PM is complete, each instrument shall have a post-PM QC check performed by QCO or designee.

5.19.2 Cleaning

5.19.2.1 When cleaning the QIAsymphony with alcohol or alcohol-based disinfectant, leave the instrument hood open to allow flammable vapors to disperse. Only clean the QIAsymphony with alcohol-based disinfectant when worktable components have cooled down.

5.19.2.2 Do not use spray bottles containing alcohol or disinfectant to clean surfaces of the QIAsymphony. Spray bottles should be used only to clean items that have been removed from the worktable.

5.19.2.3 Daily Cleaning: See Procedure for DNA Extraction for daily cleaning tasks.

5.19.2.4 Weekly Cleaning:

5.19.2.4.1 Clean Pipetting System Tip Guards: Open the "Tools" screen and Press "Maintenance SP". Move the robotic arm to the cleaning position by pressing "Tip Guards". Remove all 4 tip guards by pushing each tip guard upward until it clicks out of place and can be removed. Soak in Decon-Quat for at least 15 minutes. Rinse with water and wipe dry with kimwipe. Replace tip guards

once dry.

5.19.2.4.2 Clean the Robotic Gripper: Wipe the robotic gripper with a lint-free cloth moistened with ethanol. Wipe with a kimwipe moistened with water and dry with kimwipe. Note: Only wipe the weight. Do not wipe the rods otherwise the ball mechanism may become jammed.

5.19.2.4.3 Clean the Optical Sensor: Wipe the window of the optical sensor with a lint-free cloth. Moistening the cloth with 70% ethanol is required.

5.19.2.4.4 Clean the Eluate Drawer Adapters: Rinse eluate drawer adapters using ethanol. Rinse with water and dry with kimwipes. Check the condition of the bar code labels and ensure that they are not scratched.

5.19.2.4.5 Clean Drawers, Deck, and Lysis Station: Remove all removable objects (tube carriers, adapters, inserts, liquid waste station/tip park station, tip disposal chute, liquid waste bottle, reagent box holder) from the drawers. Wipe the drawers and lysis station with ethanol or ethanol-based disinfectant. Wipe with a kimwipe moistened with water. Dry with a clean kimwipe. Clean the top plate of the piercing device.

5.19.2.4.6 Clean the Magnetic-Head Guards: Open the “Maintenance SP” menu and run the service protocol “Magnetic head guards”. Gently raise the catches to release the magnetic-head guards. Wipe the magnetic-head guards with ethanol or ethanol-based disinfectant. Wipe with a kimwipe moistened with water. Wipe dry with a clean kimwipe. Replace the magnetic-head guards. Open the “Maintenance SP” menu and run the service protocol “Open magnetic head guards”.

5.19.2.4.7 UV Decontamination:

5.19.1.4.7.1 Remove all removable objects (tubes/plates, adapters, consumables, tip disposable chute) except for the liquid waste bottle from the drawers.

5.19.1.4.7.2 Enter the “Maintenance” screen. Press “Tools”, then press “Maintenance SP”.

5.19.1.4.7.3 To start the UV cleanup procedure, press the “Start UV light SP” button.

5.19.1.4.7.4 Set the UV decontamination time to 30 minutes.

5.19.1.4.7.5 Confirm that all removable objects have been removed from the worktable by pressing “Ok”.

5.19.1.4.7.6 Press "Ok" to start the UV irradiation procedure.

5.19.1.4.7.7 Replace all removable objects once UV procedure has been completed.

5.19.1.4.8 Reboot instrument.

5.19.1.5 Monthly Cleaning:

5.19.1.5.1 Change Tip Adapter O-Ring: Refer to the quick guide that is equipped with the O-Ring Change Tool Set to replace the tip adapter O-rings.

5.19.1.5.2 Clean Tube Carriers and Inserts: Remove tube carriers, adapters, and inserts and soak them in disinfectant (e.g., Decon-Quat). Incubate for at least 15 minutes, then rinse with water and dry with kimwipes. Check the condition of the bar code labels and ensure that they are not scratched.

5.19.1.5.3 Clean the Hood: Wipe the surface with a soft lint-free cloth moistened with deionized water. Then wipe dry with a kimwipe. Note: Do not use ethanol or ethanol-based disinfectant on the hood.

5.19.1.5.4 Clean the Touchscreen: Wipe the touchscreen with ethanol. Then wipe the touchscreen with kimwipe moistened with water. Wipe dry with kimwipe.

5.19.1.5.5 Clean the Tip Disposal Chute: Remove the tip disposal chute from the "Waste" drawer. Soak in Decon-Quart for at least 15 minutes. Rinse with water and wipe dry with a kimwipe. Replace tip disposal chute once dry.

5.19.1.5.6 Clean the Magnetic Head: Remove the cover from the magnetic head. Move the magnetic head up and carefully push the rod cover holder down. Wipe the exterior of the magnetic head with a kimwipe moistened with ethanol. Wipe with a kimwipe moistened with water and dry with a clean kimwipe. Note: insert the kimwipe from the sides of the magnetic head in order not to damage the electronic board at the front.

5.19.1.5.7 Clean Conveyor Base Tray: Carefully remove the conveyor base tray from below the magnetic head. Soak in Decon-Quat for at least 15 minutes. Rinse with water and wipe dry with kimwipe. Replace tray when dry. Note: The tray can also be autoclaved at 121 °C for 20 minutes.

5.19.3 Post PM/Repair QC Check: Before any validated Qiagen QIAsymphony may be used for casework analysis following repair or annual PM, a QC check shall be performed as follows:

5.19.3.1 At least one protocol run using CW 200, CW500, or Reference500 Assay Control Set shall be performed according to the Procedure for DNA Extraction. Alternatively, a water run utilizing the installation kit may be performed.

5.19.3.1.1 To perform a water run utilizing the installation kit:

5.19.3.1.1.1 Place the deep well EMTR plate present in the installation kit into slot 2 of the Eluate drawer. This plate does not require an adaptor.

5.19.3.1.1.2 Using the reagent rack present in the installation kit, ensure the first reagent trough is no more than ¾ full of water, then place reagent rack onto instrument.

5.19.3.1.1.3 Fill 24 large tubes with water, place in sample carrier and insert carrier onto instrument.

5.19.3.1.1.4 Begin the installation protocol run by assigning the "Installation v4" protocol and following the prompts to being the water run.

5.19.3.1.2 The QC runs are deemed to be passing/successful is they complete with no errors noted by the instrument. If samples are run quantification may be performed to check results.

5.19.3.2 After repair or preventative maintenance, the QCO shall review documentation records provided by the engineer. All testing specifications listed within the documentation shall be marked as "Pass" and the operation verification tests should be approved by the engineer. This documentation will be retained for maintenance records.

6.0 Limitations - As noted in **5.0**.

7.0 Safety

7.1 Thermal cyclers can exceed temperatures of 100 °C; use with caution to avoid burns.

7.2 Gloves, masks, and lab coats shall be worn when performing any verifications, calibrations, or QC checks described in Section 5.

7.3 If the ice shaver (or equivalent) used is not self-contained, safety glasses shall be worn during operation.

7.4 Formamide is a known chemical hazard; it causes eye, skin and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Wear appropriate eyewear, masks, gloves and clothing when in use.

7.5 This document references techniques that are written in additional detail in specific Forensic Biology Section documents. To see safety hazards for particular procedures, refer to that specific procedure.

7.6 Refer to Appendix 1 for chemical hygiene and safety precautions for extremely hazardous and particularly hazardous substances.

8.0 References

Forensic Biology Section Procedure for Use of the3500 Genetic Analyzer for Casework Forensic

Biology Section Procedure for DNA Reagent Preparation and Quality Forensic Biology Section

Procedure for DNA Quantitation with Quantifiler® Trio

Forensic Biology Section Procedure for PCR Amplification using PowerPlex® Fusion 6C for Casework

Forensic Biology Section Procedure for PCR Amplification using PowerPlex® Y23

Forensic Biology Section Procedure for Aseptic Technique and Contamination Control

Procedure for DNA Extraction

Instrument manuals

Applied Biosystems 3500 Genetic Analyzers. User Bulletin. 2005 Applied Biosystems. Part Number 4363787. Rev A. (or most recent revision)

Eutechnics 4500 Manual NIST

Special Publication 819

Applied Biosystems QS5 Fast Real Time PCR Systems. Maintenance Guide. *(Publication # MAN0017162 Rev A.0, 6/15/2017*

9.0 Records

- Temperature logs for freezers, refrigerators (Daily and Weekly)
- Thermal Cycler Temperature Performance Check Forms
- Bulb Thermometer Calibration Forms
- Biosafety Cabinets/Chemical Fume Hoods/Lamina Flow Clean Air Benches Certificates
- Certificates of Calibration for NIST Traceable Digital Thermometer, Digital Thermometers, Balances, Pipettors, Digital Probes, Weights, and Temperature Chart Recorders/Data Loggers
- Manufacturer documentation of preventative maintenance and/or repair for 3500, AB QS5 , EZ1s, QIAgilities, centrifuges
- EZ1 & EZ2 Cleaning/Maintenance Form
- QIAgility/QIACube Maintenance Forms
- QIAsymphony Maintenance Form
- 3500 Monthly Maintenance Form

10.0 Attachments

10.1 Appendix 1 – Chemical Hygiene and Safety Precautions for Extremely Hazardous and Particularly Hazardous Substances

Revision History		
Effective Date	Version Number	Reason
05/24/2024	19	5.1.3.1 – added RNaseP plate for QS5 QC; 5.3.1.2, 5.3.1.3 – updated temperature settings, fixed spacing in section; 5.12.1 update temperature recording to match lab-wide procedure; 5.19 – add PM and QC steps for QIAsymphony instrument.

Appendix 1 – Chemical Hygiene and Safety Precautions for Extremely Hazardous and Particularly Hazardous Substances

Hi-Di Formamide **DANGER: PARTICULARLY 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).