

Forensic Biology General Lab Maintenance Manual

Effective:1/12/2026

Version: 15.0

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Section 1 Chemicals and Reagents

1.1 Introduction

By definition, “critical reagents are determined by empirical studies or routine practice to require testing on established samples before use on evidentiary or casework reference samples” (FBI QAS, 2009). Reagents which are used in pre-amplification procedures directly involved in DNA extraction from forensic casework or database samples, have been deemed critical reagents to prevent unnecessary loss of samples. Except for allelic ladders, all post-amplification DNA reagents are hereby listed as non-critical reagents; allelic ladders are critical reagents. Non-critical DNA reagents need not be verified prior to use in casework.

When a reagent fails to meet the criteria for verification, the DNA Technical Manager shall be notified, and an appropriate course of action will be determined. The reagent shall not be used in casework unless or until the issue has been resolved and the approval or an alternate course of action suggested by the DNA Technical Manager has been documented.

1.2 General Instructions

- Chemical and reagent quantities may be adjusted to prepare more or less than the specified amount.
- All critical reagents prepared in-house shall be stored in sterile/autoclaved containers.
- Reagent containers are to be labeled with the following:
 - Name of reagent
 - Lot number (the date of preparation and preparer’s 2 or 3 letter initials are used as the lot # for reagents prepared in-house and reagents where a lot # is not provided by the commercial vendor, i.e., 06-0101MLC would be the lot # for a reagent prepared on Jan. 1, 2006, by MLC)
 - Expiration date
 - Reagents prepared or removed from their primary container for daily use need only be labeled with the identity of the reagent and the date and initials of the scientist that prepared or is using the reagent.
- One member of the DNA discipline shall be designated for purchasing supplies and reagents. Analysts and technicians are responsible for identifying supplies and reagents (they use) that need to be ordered and alerting the designated purchasing agent.
- Approximately once per month, a technician will confirm that the most current SDS is on file (for recently received reagents/chemicals) in the location designated in the Health and Safety Manual.
- **All** chemicals, reagents and casework supply prepared or purchased shall be logged in the reagent Log maintained in SharePoint.
 - LIMS-DNA reagents are logged into LIMS-DNA with “VERIFICATION-“, or similar, in front of the lot number (Refer to Entering and Verifying Reagents” in LIMS work instructions.) Following verification, the “VERIFICATION-“ is removed from the reagent lot number in LIMS-DNA.

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- Reagents may require logging in to JusticeTrax or LIMS-DNA as well as SharePoint – see following chart:

JT and SharePoint	LIMS-DNA and SharePoint	SharePoint Only
AP Spot Test	G2 Buffer	DTT powder
PSA Cards	MTL Buffer	un-hydrated cRNA
Hematrace cards	GTD *	GlobalFiler Express Kits
Permout	EZ1/2 cartridges	Prep-N-Go buffer
Christmas Tree Stains	DTT Solution	All Rapid Reagents
Sterile Water	cRNA solution	
Kastle Meyer	Proteinase K	Other chemicals that are used to make reagents or perform maintenance
Xylene Substitute	GlobalFiler Kits *	
Reagent Alcohol	Formamide	
	PowerPlex Y23 kits *	
	Quantifiler Trio kits	
	TE buffer	
	Sterile Water	
	GeneScan 600 LIZ	
	Sodium Acetate	
	EDTA	
	Ethanol for bone extraction	

*entered into LIMS-DNA by person verifying (GF/PPY23) or rehydrating (GTD) reagent

- All purchased chemicals/reagents are assigned the expiration date specified by the manufacturer. If no manufacturer expiration date is provided, the following guidelines apply:
 - Chemicals used in the in-house preparation of a reagent are not assigned an expiration date. Expiration dates are assigned to the prepared reagents as specified below.
 - Reagents used as received will expire one year from the date of receipt.
- Some reagents have a vendor-designated expiration date in their frozen (or dehydrated) state but then are assigned another expiration based on thaw (or rehydration date. Refer to LIMS-DNA Work Instructions for guidance on designation of expiration dates and lot numbers when multiple thaw dates affect a given lot number.
- All newly received/prepared critical reagents and chemicals shall be verified prior to use on casework/database samples. Chemicals/reagents requiring verification should be clearly marked as such.

1.2.1 Special handling considerations for STR and Y-STR PCR kits

As per vendor communications, it is possible that the outer packaging of PCR kits could potentially be contaminated with allelic ladder. To minimize the risk of introducing allelic ladder contamination into pre-PCR workspaces, additional precautions must be used when receiving and un-packaging PCR kit components.

- Disassembly of the PCR kit must take place in the vestibule outside the PCR room.

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- Disposable gloves must be worn and changed frequently during the kit disassembly process.
- Avoid placing the kit in direct contact with table surfaces – use a disposable barrier (i.e. bench paper or bench towels) to cover the bench top.
- The outer layer of clear plastic wrapping should be treated as if it could be contaminated with allelic ladder. Remove the plastic wrapping with the following procedure:
 - Use single-use scalpel and forceps to open and peel back the plastic
 - Discard scalpel and forceps
 - Change gloves
 - Remove the inside Pre-PCR box(es) to a separate covered bench area , making every attempt to avoid contact with the outer surface of the plastic wrapping
 - Discard plastic wrap
 - Change gloves

Pre-PCR reagents (Master Mix, Primer Mix, 007 Control DNA):

- Set up a tube storage box on a clean bench paper
- If the pre-PCR reagents are in an additional layer of plastic wrap, follow the above procedure for removing the next layer of plastic wrap.
- Open the box of pre-PCR reagents
- Change gloves
- Minimizing contact with packaging, remove reagents into the tube storage box.
- Change gloves
- Close the tube storage box, label, and move it to its correct storage location
- Discard packaging materials and bench paper; use bleach to clean the work surface; change gloves

Post-PCR reagents (Allelic ladder):

- Set up areas inside the PCR room for further handling, including one bench paper for removal of plastic wrap and an adjacent area for receiving the inner contents
- Remove gloves before returning to vestibule; put on clean gloves in vestibule
- Bring the post-PCR reagents into the PCR room.
- Change gloves
- Use the plastic wrapping removal procedure described above
- Open the box containing tubes of allelic ladder
- Change gloves
- Minimizing contact with packaging, remove tubes of allelic ladder into a tube storage box.
- Change gloves
- Close the tube storage box, label, and move it to its correct storage location
- Discard packaging materials and bench paper; use bleach to clean work surface; change gloves

1.3 Chemicals and Reagents not Requiring In-House Preparation and/or Verification

Chemicals/Reagents purchased from a commercial vendor and requiring no preparation or verification prior to use in procedures or preparation of other reagents are listed below. They shall be stored as prescribed by the manufacturer and shall expire on the date provided by the manufacturer. Expiration

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dates are assigned as previously described, if not provided by the manufacturer and unless stated otherwise.

- RT PCR RNase P plate [liquid]
- Anode Buffer Container, 3500 series from Life Technologies [liquid]
- AP Spot Test [solid]
- Blood standard [liquid]
- Bleach [liquid]
- Cathode Buffer Container, 3500 series from Life Technologies [liquid]
- Conditioning Reagent, 3500 series from Life Technologies [liquid]
- Dithiothreitol [solid]
- Ethanol, anhydrous reagent grade [liquid]
- GeneScan 600 Liz Size Standard [liquid]
- Hydrogen Peroxide [liquid]
- Multi-Capillary DS-36 Matrix Standards (Dye Set J6) [liquid]
- Multi-Capillary DS-33 Matrix Standards (Dye Set G5) [liquid]
- POP-4 Polymer from Life Technologies [liquid]
- Promega Matrix 5C Standard
- Semen Standard [liquid]
- Sterikon® plus Bioindicator [ampules]
- Xmas Tree Stain [liquid]
- Xylene Substitute [liquid]

1.4 Preparation and Verification

- Verifications can only be performed by trained analysts or technicians; data interpretation must be performed by a qualified analyst or the DNA Technical Manager, as described on the relevant [Reagent Verification Form](#).
- Vendor supplied standard samples / positive control samples that are sent with PCR amplification kits may be discontinued or substituted at vendors' discretion. The identity of positive control samples used in analysis will be recorded in database paperwork, serology bench notes, or in LIMS-DNA for DNA casework, as applicable.
- Similarly, variations in vendor supplied materials (changes instituted by the vendor and outside of laboratory control) will be assessed to determine if the change adversely affects the laboratory analysis in which the reagent/chemical is used. This assessment is documented in the verification paperwork. Kit component information in the chemicals and reagents section will be updated as required when the manual is revised.
- Verification of a reagent that is only used as a component of another reagent is achieved by verifying the final preparation and does not need to be documented separately.
- Reagents used in the same procedure may be verified simultaneously. If the verification fails, the components will then need to be verified separately.
- Verification paperwork is maintained by calendar year in SharePoint and shall include the appropriate [Reagent Verification Form](#).
- For successful verification of screening reagents, the positive and negative controls must perform as described in the Forensic Biology Casework Procedures Manual. Reagents must be successfully verified prior to use in casework.

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- Verification must be performed using the most stringent conditions routinely encountered in casework, including GenTegra dry-down and re-hydration in minimum amplification volume where applicable.
- For verifications that include amplification and electrophoresis, the paperwork consists of the electropherograms for the positive control/reference sample(s) and negative control/blank(s). Verification results are assessed as described in the Interpretation section of the Forensic Biology Casework Procedures Manual. The expected results must be obtained for a chemical/reagent to be successfully verified and appropriate for use in casework/database analysis.
- For verifications that include peak height assessments, a copy of the peak height assessment must be included in the verification documentation.
- In the verification of casework amplification kits, the relative fluorescence units (RFU) for the known sample amplified with the new kit are compared to the results obtained with the kit currently in use to estimate the sensitivity of the new kit. This is important for adjusting the target value with the new lot of kits.
- Control documentation for verifications may be referenced by noting the database batch or LIMS-DNA set name in which the verification was performed.
- Upon successful verification, the verification paperwork is stored in [SharePoint](#).
 - Reagents are logged into JusticeTrax or LIMS-DNA as per the [Reagent Verification Form](#).
- When verification fails on a reagent prepared in-house, the reagent may be re-prepared and/or verification repeated. If verification fails again, consult with the DNA Technical Manager to determine the appropriate course of action. For purchased reagents/chemicals, the DNA Technical Manager shall be consulted to determine the appropriate course of action.

ABAcad® p30

Purchased from Abacus Diagnostics. Stored according to manufacturer's instructions.

Verification

The verification process is described and documented on the ABAcad® p30 for the Forensic Identification of Semen [Reagent Verification Form](#).

ABAcad® HemaTrace®

Purchased from Abacus Diagnostics. Stored according to manufacturer's instructions.

Verification

The verification process is described and documented on the One-Step HemaTrace ABAcards [Reagent Verification Form](#).

AP Spot Test

Purchased from SERI. Aliquot (0.13 g in 15 mL or 50 mL foil-wrapped conical tube) and store at -15 to -20° C. Expiration for frozen powder is manufacturer's expiration date. Expiration date is one day from date of working solution preparation.

Working solution

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Add 5.0 mL of dH₂O to 0.13 grams of AP Spot Test. Mix the solution thoroughly and remove from foil briefly to ensure all powder is dissolved. Store the working solution at room temperature protected from light (e.g., wrap the container in aluminum foil). Cap the working solution when not in use to minimize oxidation.

Note: Different volumes of working solution may be prepared if the ratio of AP Spot Test to dH₂O remains constant.

Verification

The verification process is described and documented on the AP Spot Test [Reagent Verification Form](#).

Buffer G2 (critical reagent)

(when purchased outside of a kit)

Purchased from Qiagen and stored at room temperature

Verification

The verification process is described on the Buffer G2 [Reagent Verification Form](#).

Buffer MTL (critical reagent)

Purchased from Qiagen and stored at room temperature

Verification

The verification process is described and documented on the Buffer MTL [Reagent Verification Form](#).

GenTegra DNA (critical reagent)

Purchased from GenTegra and stored at room temperature prior to hydration, stored at 2-8°C following hydration. Expiration date of the dried form is manufacturer's expiration date. Expiration date of the hydrated GenTegra-DNA stock is three months after prep date. Typically, several tubes of Gentegra LD are rehydrated on the same date and given a sub-lot designation in LIMS-DNA.

Verification

The verification process is described and documented on the GenTegra-DNA [Reagent Verification Form](#).

DTT 1M (critical reagent)

Prior to being used to make a working solution, dry DTT powder is stored at 2-8°C.

Working Solution Dissolve 0.77g dithiothreitol in 5mL sterile de-ionized water in a sterile conical tube. Add 50µL of 3M Sodium Acetate buffer solution, pH 5.2. Do not autoclave. Formula may be scaled up to prepare larger quantity. Aliquot (0.5 – 1.0 mL recommended) and store at -15 to -25°C. Aliquots expire one year from date of preparation. Typically, an entire DTT batch is considered thawed during verification so that there is one expiration date for the entire lot. DTT is stored at -15 to -25°C even after first thaw.

Verification

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The verification process is described and documented on the DTT [Reagent Verification Form](#).

EDTA, 0.5M, pH 8.0 (critical reagent)

Purchased from VWR or other suitable vendor, aliquoted, and stored at room temperature.

Verification

The verification process is described and documented on the EDTA [Reagent Verification Form](#).

Ethanol (for bone extraction), denatured, anhydrous, 94-96% (critical reagent)

Purchased from VWR or other suitable vendor, aliquoted, and stored at room temperature.

Verification

The verification process is described and documented on the ethanol [Reagent Verification Form](#).

EZ1/EZ2 DNA Investigator Kit (critical reagent)

Components: Reagent Cartridges, Buffer G2, Proteinase K solution, carrier RNA

Purchased from Qiagen and stored at room temperature.

Carrier RNA solution is prepared by reconstituting the carrier RNA in 310µL of sterile, de-ionized water. Vortex and spin briefly. Prepare 20µl, single use aliquots in 0.5mL tubes and store at -15 to -25°C. Reconstituted carrier RNA expires one year from date of preparation.

Verification

The verification process is described and documented on the EZ1/EZ2 DNA Investigator Kit [Reagent Verification Form](#).

GlobalFiler Amplification and Typing Kit (critical reagent)

Components: GlobalFiler Master Mix, GlobalFiler Primer Set, GlobalFiler Allelic Ladder, DNA Control 007

Purchased from Applied Biosystems. Stored at -15 to -25°C upon receipt and until verification, stored at 2 to 8°C after initial thaw for up to 6 months or up to the expiration date stated on the kit (whichever comes first). Typically, approximately ½ the tubes in a large lot (~ nine or more kits) are thawed during verification. The second ½ are thawed as a “sublot” when needed. For lots of 8 or fewer kits, the entire lot may be thawed at verification.

Verification

- The verification procedure is detailed and documented on the GlobalFiler [Reagent Verification Form](#).
- Results must be submitted to the Technical Manager for approval of the kit; this is accomplished by assigning review of the verification to the DNA Technical Manager in LIMS-DNA. Average peak height must be within validation range specific on verification sheet, and peak height

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variation between old and new kits should be within 30%. The DNA Technical Manager will monitor performance among GlobalFiler kit lots.

- The course of action for a kit that fails verification will be determined by the Technical Manager.

GlobalFiler Express Kit (critical reagent)

Purchased from Life Technologies and stored at -15 to -25°C until thawed, then stored at 2-8 °C. Expiration date is either six months from date of thaw or manufacturer's expiration date, whichever comes first.

Components: DNA Control 007, Master Mix, Master Mix Additive, Primer Set and GlobalFiler Express Allelic Ladder.

Verification Procedure

The verification process is described and documented on the GlobalFiler Express and/or Prep-N-Go [Reagent Verification Form](#).

Hi-Di Formamide

Purchased from Life Technologies. Aliquot (0.5mL and 1.0mL recommended) and store at -15 to -25°C. Aliquots are intended for one-time use and should not be re-frozen.

Internal Control Samples (ICS - for extraction sets and performance checks)

It is recommended that different types of ICS samples are distinguished by using different color tube caps and that each newly prepared set of ICS samples are placed into a new rack, rather than adding to an existing rack of samples. Tubes should be labeled on the side with the contents. This can be abbreviated but should allow for easy identification of the expected profiles. Use of the Tubewriter will facilitate the labeling process.

Direct – recommended to use a male source so that the sample is suitable for use with Y-STRs

- Aliquot a volume of single source blood to allow for 20uL per sample

NOTE: Do not pipette onto the swabs directly from the stock solutions. Use of a swab rack will facilitate the next steps.

- Pipette 20uL on blood onto each swab
- Let the swabs air dry in a hood.
- Snap one entire swab head into each pre-labeled tube.
- Label the completed rack and place in the designated storage location.

Direct w/DTT – alternatively, a differential ICS may be used for this type of extraction set

- Aliquot a volume of single source semen to allow for 15uL per sample

NOTE: Do not pipette onto the swabs directly from the stock solutions. Use of a swab rack will facilitate the next steps.

- Pipette 15uL of semen onto each swab
- Let the swabs air dry in a hood.
- Snap one entire swab head into each pre-labeled tube.
- Label the completed rack and place in the designated storage location.

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Differential

- Aliquot a volume of single source semen to allow for 15uL per sample
- Aliquot a volume of single source female blood to allow for 20uL per sample

NOTE: Do not pipette onto the swabs directly from the stock solutions. Use of a swab rack will facilitate the next steps.

- Pipette 15uL of semen onto each swab
- Let the swabs sit long enough for the semen to be absorbed.
- Pipette 20uL on blood onto each swab
- Let the swabs air dry in a hood.
- Snap one entire swab head into each pre-labeled tube.
- Label the completed rack and place in the designated storage location.

NOTE: Alternatively, a freshly collected buccal swab (preferred male for direct and preferred female for differential) may be used to prepare ICS samples when a liquid blood standard is not available. If using a buccal for Direct w/DTT or Differential ICS', the semen may be spotted directly on the buccal swab.

Permout

Purchased from a commercial vendor and stored at room temperature.

Working Solution: Permout diluted with Xylene substitute if necessary. Use until no longer functioning adequately as a mounting medium.

Phenolphthalein Presumptive Blood Test Kit

Purchased from Arrowhead Forensics and stored at room temperature.

Verification

The verification process is described and documented on the Phenolphthalein Presumptive Blood Test Kit [Reagent Verification Form](#).

PowerPlex Y23 Kit (critical reagent)

Purchased from Promega and all except 2800 control DNA are stored at -15 to -25°C until thawed, then stored at 2-8 °C. 2800 control DNA is always stored at 2-8 °C. Expiration date is either one year from date of thaw or manufacturer's expiration date, whichever comes first. Typically, all tubes in the lot are thawed during verification so that there is one expiration date for the entire lot.

Components: DNA Control 2800, Master Mix, Primer Set, WEN ILS 500 Y23, and PowerPlex Y23 Allelic Ladder.

Verification

- The verification procedure is detailed and documented on the PowerPlex Y23 [Reagent Verification Form](#).
- Results must be submitted to the Technical Manager for approval of the kit; this is accomplished by assigning review of the verification to the DNA Technical Manager in LIMS-DNA. Average

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peak height must be within validation range specific on verification sheet, and peak height variation between old and new kits should be within 30%.

- The course of action for a kit that fails verification will be determined by the Technical Manager.

Prep-N-Go Buffer (critical reagent)

Purchased from Life Technologies and stored at room temperature.

Verification

The verification process is described and documented on the GlobalFiler Express and/or Prep-N-Go Reagent Verification Form.

Proteinase K Solution (critical reagent)

(when purchased outside of a kit)

Purchased from Qiagen or another suitable vendor and stored at room temperature

Verification

The verification process is described and documented on the Proteinase K [Reagent Verification Form](#).

Quantifiler Trio Kit (critical reagent)

Components: PCR Reaction Mix, Primer, DNA Standard, Dilution Buffer

Purchased from Life Technologies. All reagents received and stored at –15 to –25 °C until thawed for first use. Once thawed, reagents are stored at 2-8 °C. Standard curves have an expiration date of two weeks after date of preparation.

Verification

- The verification procedure is detailed on the Quantifiler Trio [Reagent Verification Form](#).
- Acceptable criteria are defined in FBPM, current version. Follow the procedure defined in that section for non-passing results.
- Document the passing T-S, T-L, and T-Y Y-intercept values in the QuantStudio 5 instrument logbook.
- Submit the Quantifiler Trio Reagent Verification Form and the Experimental Results Report to the DNA Technical Manager for approval.

RapidHIT ID Primary Cartridge and Controls (critical reagent)

Components: Primary cartridge, gel cartridge, ACE GFE Control cartridge, ACE GFE positive control cartridge, negative control cartridge

Purchased from Applied Biosystems. Primary cartridge stored at room temperature; other reagents stored at 4-10° C. Expiration date for the installed primary cartridge is six months from date of install, the expiration date of the gel cartridge, or the expiration date of the primary cartridge – whichever of these three comes first.

Verification

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After installation of the primary cartridge, run an allelic ladder (control cartridge), a positive control, and a negative control. Complete the Primary Cartridge Change Form and retain with electropherograms of allelic ladder, positive control, and negative control.

RapidHIT ID ACE GFE Sample Cartridges (critical reagent)

Components: Sample cartridges, positive control cartridges (verified along with primary cartridge), negative control cartridges

Purchased from Applied Biosystems and stored at 4-10° C. Expiration date is set by manufacturer.

Verification

Analyze a previously typed reference buccal swab using a sample cartridge from the new lot and analyze a negative control cartridge. Complete the RapidHIT ACE GFE Sample Cartridges Verification Review Form.

Sodium Acetate, 3.0M, pH 5.2 (critical reagent)

Purchased from VWR or other suitable vendor, aliquoted, and stored at room temperature.

Verification

The verification process is described and documented on the sodium acetate [Reagent Verification Form](#).

Sterile De-ionized Water (H₂O) (critical reagent)

Option 1: Prepare in house: Fill glass bottles with nanopure de-ionized H₂O. Autoclave (alongside a Sterikon™ plus Bio-indicator, or equivalent) for 30 minutes and store at room temperature. Expires 1 year from date prepared.

The autoclaved ampoule and a control ampoule that are placed in an incubator (at ~56°C) for a minimum of 48 hours. Evaluate as per manufacturer's instructions. Seek Technical Manager guidance when the autoclaved ampoule does not perform as expected.

The DNA Technical Manager must approve use of reagents autoclaved without a Sterikon™ (or equivalent). This approval will be documented in the Reagent Log.

Option 2: Purchased from VWR or other suitable vendor and stored at room temperature

Verification

The verification process is described and documented on the Sterile Water [Reagent Verification Form](#).

TE -4 Buffer (critical reagent)

Purchased from Life Technologies, aliquoted, and stored at room temperature. May be labeled by vendor as DNA Suspension Buffer.

Verification

The verification process is described and documented on the TE⁻⁴ (DNA Suspension) Buffer [Reagent Verification Form](#).

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Section 2 General Laboratory Maintenance and Environmental Conditions

2.1 General Laboratory Maintenance

The Forensic Biology analysts and technicians are responsible for the laboratory's housekeeping and the routine maintenance of equipment and instruments. Tasks are delegated as below, but all discipline members may assist as needed. Logs are completed as appropriate.

- Receipt of packages and logging of chemicals/reagents. **(Technician)**
 - Indicate date received on packing slip, initial and provide to the unit supervisor. Not all shipments contain packing slips.
 - Unpack contents, label with date received and initials, store them in the proper location, record in log and LIMS-DNA, if applicable.
 - Label with “needs verification” stickers and note (in designated locations) that verification is required (if appropriate)
- Inventory and restocking of labs
 - Gloves and masks in vestibules **(Technician)**
 - Casework reagents/supplies in lab spaces **(Analysts)**
 - Reagents/chemicals required for instrument maintenance and PCs **(Technician)**
- Autoclave water (when not available for purchase). **(Technician)**
- Autoclave consumables (tubes, toothpicks, etc.) approximately weekly **(Technician)**
- Prepare in-house reagents, ICS’ **(qualified Technicians, Analysts if needed)**
- Make sure that new kits/reagents are verified in a timely fashion. **(qualified Technicians, Analysts if needed)**
- Clean laboratory common spaces as needed (post-PCR lab, Rapid room, reagent prep room, and lab vestibules cleaned every three months at a minimum, all other labs monthly at a minimum), wiping down counters, computers, centrifuges, phones, door handles, etc. with 10% bleach. **(Technician)**
 - **All staff** are responsible for bleaching a workspace after use, cleaning and putting away dishes, packing up full biohazard/trash
 - **Technicians** will transfer full biohazard to designated location and retrieve new boxes
- Sweep and mop floors as needed (every two months at a minimum) **(Technician)**
- Bleach wipe down of communal safety hoods (every three months at a minimum) **(Technician)**
- UV PCR set-up hoods for 30 minutes after use. **(All staff)**
- Wipe down equipment/instruments as used **(All staff)**
- Adding new log sheets to maintenance and temperature log binders **(All staff)**
- Reboot genetic analyzer computers weekly. **(All staff, prior to 1st use of week)**
- Perform weekly, four-week, quarterly and semi-annual maintenance on instruments. **(Technician)**
- Replenish reagents on genetic analyzers, as needed. **(All staff)**

2.1.1 Special considerations: cleaning bone extraction area and equipment

Cleaning, sampling, and pulverizing bones happen in the designated area of the ALS room. Bone powder presents unique cleaning concerns, both because bleach does not destroy DNA contained inside the bone powder and because the dry climate and static electricity mean the bone powder can disperse

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readily. Refer to the bone extraction protocol in the Forensic Biology Procedure Manual for more details specific to workspaces used in the initial handling of bone powder. NOTE: Once reagents have been added to the bone powder, the tubes containing bone powder can be handled in other workspaces using standard cleaning protocols.

2.2 Environmental Conditions

Based on vendor recommendations for the use of EZ1/2 Investigator Kits and POP-4, ambient temperature in extraction and post-amplification lab spaces must be in the range of 15 °C to 25 °C while analysis is being performed. These lab spaces are monitored using the lab-wide temperature monitoring system (also used to monitor refrigerators and freezers as applicable), and a designated supervisor is notified when the room temperature falls outside the acceptable range.

Many of the instruments used in the Forensic Biology discipline rely on the calibration of the X/Y/Z axes and optical components. During periods of extreme seismic activity, these components may become misaligned, adversely affecting the instrument's function. Additional performance checks may be appropriate before resuming use of these instruments following seismic activity. These determinations will be made by discipline management, as needed.

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Section 3 Equipment / Instrument Maintenance

All maintenance and performance check records are maintained with the instrument, unless otherwise specified. Each calendar year, records are archived in the [Equipment Records](#) in SharePoint. Week 1 is the week which contains the first working day of the new year. It may include days from the previous year.

When laboratory equipment is placed out of service for any reason, a note will be made in the equipment/instrument maintenance log (if applicable, as not all equipment has a maintenance log) and the equipment clearly marked with a note to alert scientists not to use the equipment until further notice. Unless otherwise noted, routine maintenance and performance checks are not required while an instrument is out of service.

Manuals referenced in this section are either available online, on the laboratory network or in the designated location in the Forensic Biology discipline. Critical equipment/instrument manuals will be retained indefinitely. Non-critical equipment/instrument manuals are not required and do not need to be retained for equipment no longer in use.

Critical equipment/instrument is noted in the sub-headings below. New items of critical equipment/instrument require a performance check before use in casework and/or database analysis.

3.1 Temperature Logs

Temperatures for refrigerators/freezer that contain chemicals, reagents and evidence are monitored electronically as a component of the laboratory security system. Temperatures for incubators are recorded by the scientists when equipment is in use.

The discipline supervisor or DNA Technical Manager will be notified (by the lab manager or maintenance specialist) if a temperature falls outside of the acceptable range. Temperatures may be out of range following a prolonged period of the unit's door being opened. If the temperature falls outside of the acceptable range and is not corrected by a later second reading or a minor adjustment of the unit's temperature control, the DNA Technical Manager is consulted to determine a course of action.

3.2 Microscopes

Reference: <http://www.leica-microsystems.com/>
Leica DM1000/Leica DM1000 LED Operating Manual

General Instructions

- Simple dust is the number one enemy of microscopes and optical quality. When the scope is not in use, it should be covered with a plastic dust cover. Never leave a tube or an objective port open so that dust can get to the internal surfaces.
- When cleaning of the microscope stand is required, use a clean, lint free cloth lightly moistened with water containing a small amount of mild detergent. Quickly follow the cleaning by wiping with a dry lint free cloth.
- Any residue of mounting medium or immersion oil on the stand or stage should be removed immediately after examinations are completed, using a cotton-tipped swab or cloth lightly moistened with xylene substitute. Following this solvent cleaning, the xylene substitute should

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be removed as quickly as possible using a clean, dry cloth. It is wise to follow the solvent removal with the above detergent cleaning.

- Before any physical contact is made with the lens surfaces (eyepieces, objectives, condenser, field diaphragm), any loose dust or debris should be blown off using compressed gas. Any stubborn dust, dirt or oil can be removed using lens cleaning fluid and a cotton-tipped swab.
- Proceed to clean the lens with a moistened swab by placing the tip at the center of the lens and working with light pressure toward the outside of the lens in a spiral motion. Immediately repeat this process using a dry swab. For very small objective lenses, the swab may be gently rotated between the thumb and forefinger while it contacts the lens. Examine the surface of the lens in reflected light for any evidence of smearing, if the surface is not completely clean repeat the process. When clean, a coated lens will have a uniform bluish color. It may be necessary to use a small amount of xylene substitute to remove oil or other mounting mediums (see above).
- Scopes should be cleaned, lubricated, and aligned, when necessary, by a competent microscope mechanic.
- If artifacts caused by dirt are seen in the microscope image, one can locate their source in the following manner:
 - If the trouble can be eliminated by a slight adjustment of the condenser, look for the cause in the lamp bulb, lamp condenser, or filter in front of it.
 - If a change of focus control eliminates the artifact, look to the condenser or specimen itself.
 - If rotation of the objective lens causes the artifact to move, the soil is obviously on the objective. Similarly, if rotation of the eyepieces causes the artifact to move, the soiling is on the eyepiece.

Operation / Troubleshooting / Maintenance

See referenced manuals.

3.3 Thermometers (critical instrument)

Thermometers and probes used for extraction lab instrument performance checks

Purchase Specifications: Immersion thermometers are purchased for use in laboratory incubators and heat blocks. The incubator thermometers must cover the temperature range of 27-60°C. The heat block thermometers must cover the temperature range of 95-99°C. The thermometers must have divisions at 1°C increments (at a minimum) and have an accuracy of +/- 1°C. These thermometers will be replaced with new thermometers after approximately two years or sooner in the event of a failing performance check.

Digital thermometers with probes are purchased for use in EZ1 and thermomixer performance checks. The thermometers must cover the temperature range of 56-90°C, with a resolution of at least 0.1°C and an accuracy of +/- 1°C, and they must be accompanied by a certificate that indicates NIST traceability from a calibration laboratory accredited to ISO 17025. These thermometers will be replaced with new thermometers when their calibration expires or after approximately one year if the certificate does not provide a calibration due date.

Bottle thermometers are purchased for use in the performance checks of the immersion thermometers. The thermometers must cover the temperature ranges of the immersion thermometers, with divisions

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at 1°C increments (at a minimum) and an accuracy of +/- 1.5°C, and they must be accompanied by a certificate or statement of accuracy and NIST traceability that from a laboratory accredited to ISO 17025. These thermometers will be replaced with new thermometers after approximately one year from purchase if the certificate does not provide a calibration due date.

Performance check: The immersion thermometers are checked prior to being placed into service and semi-annually thereafter against a NIST-traceable thermometer. Passing results are within +/- 5°C of the NIST-traceable thermometer.

Unacceptable data: Thermometers with PC results outside range, as well as broken or otherwise non-functional thermometers, are discarded and replaced.

Documentation:

- Labeled with date due for next PC, calibration or replacement
- Inventory: tracked in Sharepoint Equipment List document library, to include the S/N, status and location
- Certificates: retained in [Equipment Records](#)
- Performance checks:
 - This information is recorded on relevant incubator or heat block log and includes the thermometer serial number, date of check against NIST-traceable thermometer, results of check (pass/fail), and analyst initials.
 - Archived annually in [Equipment Records](#)

3.4 Mettler Toledo XS204 Analytical Balance (or similar) and Reference Mass Set

Reference: Excellence Analytical Balances XS Models Operating Instructions

Analytical balances are performance checked quarterly. Calibration and/or service will only be performed in the event of a failed performance check or other mechanical failure. The reference mass set used for the performance checks is calibrated annually as per specifications in the [Seized Drug Procedure Manual](#). Since the balance performance check only uses reference masses between 1g and 10g, failures in the reference set to other weights do not invalidate the use of the set for the biology balance performance checks.

The quarterly performance checks are generally performed in January and every 3rd month thereafter and are recorded on the [Balance Performance Check](#) form. Records of performance checks, calibration and service are archived annually in SharePoint.

When a balance calibration is performed in the same month as the required performance check or in the month prior to it, the calibration may serve in lieu of the quarterly balance check. If performed in the same month, the calibration/PC must be completed prior to use of the balance for any EZ1 semi-annual PCs that are occurring during that month.

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3.5 Qiagen BioRobots (critical instrument)**3.5.1 Qiagen BioRobot EZ1 Advanced-XL***Reference: EZ1 Advanced XL User Manual**Qiagen supplementary protocol MA67 (Evaluating pipetting accuracy of the EZ1® Advanced XL using the EZ1 Advanced XL Test Card)**Qiagen supplementary protocol MA68 (Evaluating the temperature accuracy of the EZ1® Advanced XL)***Maintenance Procedures**

Preventive Maintenance procedures are described in Section 6 of the EZ1 Advanced XL User Manual and recorded on the log (see EZ1 Maintenance Forms).

3.5.1.1 Regular in-use maintenance

Regular maintenance is performed after each run and includes cleaning the piercing unit and wiping down work surfaces in the instrument (reference 6.1 in User Manual). Daily maintenance is performed at the end of each day the robot is in use and includes inspecting the O-rings to ensure they are clean and intact, 30-minute UV decontamination as described in Section 5.7 of the User Manual, and wiping down the instrument (reference 6.2 in User Manual). Note: It is only necessary to clean other interior surfaces in the EZ1 with dilute neutral soap solution in instances where a spill or splashing has occurred.

NOTE: EZ1 Advanced XL instruments have a UV lamp life of 1500 cycles. The instrument will give a warning when the lamp needs to be replaced (50 cycles remaining). Notify the discipline supervisor if this warning is received.

O-rings will be greased (refer to section 6.3 of the User Manual) in week 3 or 4 of a 4-week cycle.

Any preventive maintenance (PM) and service to the instrument, as well as the dates that an instrument is taken out of service or returned to service are also recorded.

3.5.1.2 Semi-annual Performance Checks

Performance checks shall be run bi-annually, regardless of whether or not service was performed on the instrument. Additionally, any instrument having PM or service performed shall be subjected to a performance check prior to being used again for casework analyses.

NOTE: Prior to performing semi-annual performance checks, ensure that semi-annual maintenance has been performed on the balance.

Procedure: Performance checks include pipetting accuracy, leakage test, and temperature accuracy. These are performed in accordance with Qiagen supplementary protocols MA67 and MA68 and are documented on the Maintenance Log.

Evaluating results:

Acceptable ranges for each test are also listed on the performance check forms.

Pipetting accuracy - 100mL of water (acceptable range 92-108mL)

Pipetting accuracy - 500mL of water (acceptable range 460-540mL)

Leakage test – no dripping

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Temperature accuracy test - measured temperature is within +/- 3°C of 60 °C

Unacceptable data: If a test fails, repeat the test. If it fails a second time, mark the instrument as offline on the instrument itself and in its logbook, and notify the DNA Technical Leader to determine a course of action.

Documentation of completion and approval/rejection: The forms for evaluating and recording the results of a performance check (four pages) are located in the EZ1 Maintenance Forms document, or as freestanding spreadsheets (which automatically perform calculations). Printouts of completed forms, including a pass/fail assessment, are kept in the EZ1 maintenance logbook until the end of the calendar year, when they are archived electronically.

3.5.2 Qiagen BioRobot EZ2 Connect (critical instrument)

Reference: EZ2 Connect and EZ2 Connect Fx User Manual

Maintenance Procedures

3.5.2.1 Regular in-use maintenance

- Remove all sample preparation waste and dispose.
- Remove eluates and store appropriately.
- Close the hood.
- Tap Move down to lower the piercing unit of the pipettor head.
- Open the hood.
- Wipe the piercing unit with a Kimwipe moistened with ethanol, followed by a Kimwipe moistened with distilled water. Note: piercing unit is sharp -use caution.
- Close the hood.
- Tap Finish to return the pipettor head to its home position.
- Clean worktable surfaces with a Kimwipe moistened with ethanol, followed by a Kimwipe moistened with distilled water. Note: the hood should only be cleaned with a Kimwipe moistened with distilled water.
- Lightly wipe D-rings with a Kimwipe to remove any residual liquid and check for damage.
- On the touchscreen, choose Maintenance, then UV Run. Select 1 decontamination cycle and press start.

3.5.2.2 Four-week maintenance

Note: The EZ2 User guide recommends weekly D-ring greasing, but previous experience with greasing D-rings has shown that four-week greasing is sufficient. Also, the daily in use maintenance includes checking the D-rings.

- D-rings will be greased during week 3 or 4 of a 4-week cycle, following the procedure below:
 1. Clean the D-rings (on tip adapter) with a lint-free tissue to remove any previously applied grease.
 2. Apply a small amount of silicon grease onto the inner wall of the large end of a fresh filter-tip by using the fine end of a second fresh tip.
 3. Place the previously prepared filter-tip with grease applied to the inner wall at the wide end onto each tip adapter subsequently and rotate the filter-tip on the tip adapters to distribute the silicon grease evenly. The same tip can be used for the distribution of grease on all D-rings. Apply new grease into the filter tip after every four tip adapters according to the previous step.

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4. Make sure that the D-rings are only moistened with grease and that there are no visible grease residues. Residues should be removed with lint-free cloth and greasing restarted.
5. Make sure that there is no grease present on parts other than the D-rings, especially on the bar on top and the opening of the pipettors.
6. Use a pipette tip to check that each pipettor is able to hold a tip without slippage.

Note: Excess or insufficient grease can affect the performance of the E22 Connect.

Note: The opening in the nub of the pipettor should be checked after greasing to ensure no grease is present within the opening.

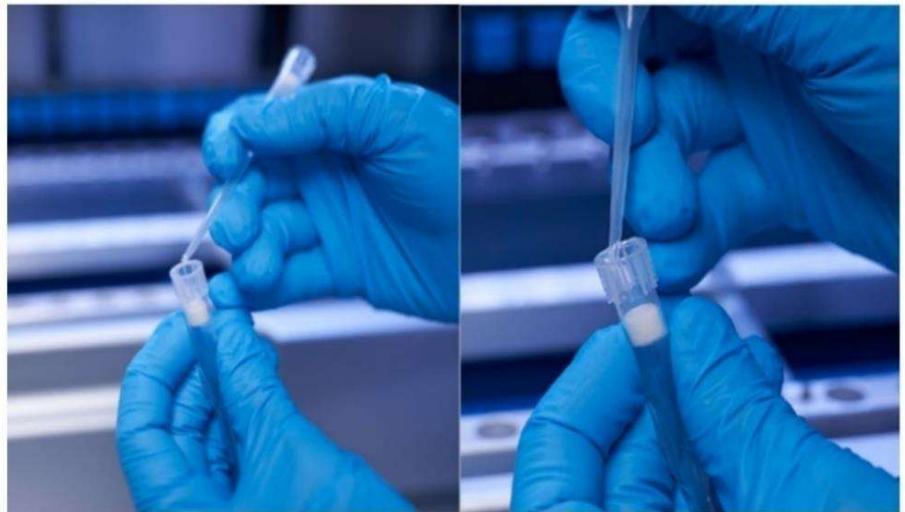
Note: E22 performance issues can arise when following the vendor-specified greasing protocol leads to pipette tip slippage. In this case, the frequency of D-ring greasing may need to be changed. At a minimum, the four-week maintenance must include checking the condition of the D-rings. Greasing is not mandatory if the D-rings still appear shiny and well lubricated. The maintenance log will be updated to indicate whether or not greasing was performed during the four-week maintenance.



Rings that appear shiny and sufficiently lubricated (as shown in this image) do not require additional greasing.

The amount of grease on the tip is the recommended amount to distribute inside the collar of the tip.

Use of gloved fingers to apply grease is not preferred.



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Example of an over greased adapter is shown.

All marked spots should be checked, and all visible grease residues should be removed.



3.5.2.3 Annual preventive maintenance

Annual preventive maintenance is performed by the vendor. Any preventive maintenance (PM) and service to the instrument, as well as the dates that an instrument is taken out of service or returned to service are recorded in the instrument log.

Performance Checks

Any instrument having PM or service performed shall be subjected to a performance check prior to being used again for casework analyses.

Procedure: A staff buccal swab or ICS sample, plus a reagent blank, will be extracted, quantified, amplified, and analyzed. Results will be evaluated by casework interpretation guidelines.

Evaluation: Extracts generated in the performance check are quantified, amplified, and analyzed. Resulting data is analyzed according to criteria described in the current version of the Forensic Biology Procedure Manual. In addition, they are checked to make sure the correct profiles were obtained. A passing performance check consists of correct and complete profiles for the positive control sample, plus an amplified reagent blank profile without extraneous DNA.

Unacceptable data: A performance check failure would be an incomplete or incorrect positive control profile, or a reagent blank profile with detected DNA. However, an incomplete profile, and some instances of reagent blank contamination, may be indicative of an issue at the amplification stage. Such samples should be re-amplified. If re-amplification does not yield a full, correct profile for a positive control sample and/or a reagent blank profile without extraneous DNA, then the performance check fails and the performance check should be re-attempted. If the second effort also fails, the instrument must be taken offline and the Technical Manager must be notified, so that a further course of action can be determined by the Technical Manager.

Documentation of completion and approval/rejection: Performance check documentation includes electropherograms of the successful extraction positive and negative controls. If the performance check was performed as a part of a casework central log, the central log may be referenced. If the performance check was performed alone, documentation such as allelic ladders, amplification controls, etc. should be included with the performance check paperwork. An electronic scan of the compiled

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performance checks documentation for a calendar year is then stored on the laboratory network at the end of the year.

3.6 Thermo-Mixer (critical instrument)

3.6.1 Operation

- Turn on main switch.
- Calibrated set points for the digital display in the thermomixer unit are checked semi-annually and noted on the instrument, with analyst date and initials. Choose digital temperature set point accordingly.
- Allow thermomixer to come to temperature, as shown on the digital display.

3.6.2 Performance Check

As a semi-annual performance check, the digital set-points for specified temperatures (i.e. 56°C, 70°C, and 90°C) will be re-assessed for both standard tubes. 2 ml Thermo-mixers will be re-assessed for both standard tubes and Lyse & Spin basket tubes.

Procedure for standard tubes:

- Put ~1000uL of sterile water in a tube and place in the thermomixer (without shaking).
- Set temperature on the digital display to the previously calibrated value noted on the instrument. Allow thermomixer to come to temperature, as shown on the digital display.
- Use a temperature probe to determine actual temperature.

Procedure for Lyse & Spin baskets (only for 2 ml Thermo-mixers):

- Cut ~half the lid off a Lyse & Spin basket to allow for use of a temperature probe.
- Put ~500uL of sterile water in the basket and place in the thermomixer (without shaking).
- Set temperature on the digital display to the previously calibrated value noted on the instrument. Allow thermomixer to come to temperature, as shown on the digital display.
- Use a temperature probe to determine actual temperature.

Evaluating results:

- If the standard tube digital set point is no longer correct but within +/- 5 °C of the temperature displayed on the screen (or within +/- 5 °C of the previous set point for Lyse & Spin basket tubes), adjust gradually until the correct set-point is found, allowing adequate time for temperature stabilization during the adjustment process.

Documenting completion and approval / rejection:

- Note any changes to the digital set-points on the instrument.
- Completion is documented on the Thermomixer Temperature Log.

Unacceptable results:

- For the standard tubes, if the temperature on the thermometer and the displayed temperature differs by more than 5 °C, or if the temperature reading will not stabilize, the instrument will be taken offline – label the instrument clearly as offline, note as offline in the maintenance log, and notify the Technical Lead

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3.7 Applied Biosystems QuantStudio 5 (QS5) Real-Time PCR System (critical instrument)*Reference: QuantStudio 5 Real-Time PCR Instrument (for Human Identification) User Guide***3.7.1 Maintenance Procedures**

Directions for performing the checks listed below are in *QuantStudio 5 Real-Time PCR Instrument (for Human Identification) User Guide*. Maintenance is recorded in the Maintenance Log. It is not necessary to perform maintenance if the instrument is not in use for the relevant time period. Record as “Not In Use” in the Maintenance Log as applicable.

Weekly in use

- Prior to the first run of the week, power off the computer controlling the QS5 instrument, then turn off the QS5 instrument. After 30 seconds, power on the instrument and computer.
- Clean the instrument outside surface with a lint-free cloth.

Four-week

- Perform an instrument self-verification test (Under Settings – Maintenance and Service – select Self Verification Test)
- Perform a background calibration to check for thermal block contamination. To create a background plate:
 - Remove a reaction plate from its box and place it on a clean, dry surface.
 - Aliquot deionized water to each well of the reaction plate. The recommended volume for a 96-well plate is 10–20 µL.
 - Seal the plate using an optical adhesive cover.
 - Use the plate for background calibration.
- Run disk cleanup and disk defragmentation on the computer.
- On the QS5 instrument, delete experiments with dates from two months prior. For example, four-week maintenance performed on July 15 would delete experiments from May and earlier.
 - Delete experiments on the QS5 instrument by going to Settings --> Run history --> Manage --> Delete (May need to log in as Admin)
 - You can click on multiple projects at a time to delete.

As needed

- Decontaminate the QS5 instruments
- Perform an RNase P instrument verification run after moving the instrument (consult with Technical Manager if uncertain as to whether this is required)
- Check computer disk space. If necessary, delete experiment files.

3.7.2 Annual Preventive Maintenance

- This is conducted and evaluated by an outside vendor, according to their protocols. It includes temperature verification, background, ROI / Uniformity calibration (only required once every two years, dye calibrations (only required once every two years), and other functionality tests. This serves as the annual performance check of those systems. Documentation consists of a completed report from the vendor technician, which is reviewed to confirm that results were passing. The report is added to the instrument maintenance logbook until it is archived electronically at the end of the calendar year.

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Performance Check following annual PM/service/repairs to instrument

Following annual preventive maintenance or service/repairs, a performance check will be run prior to using the instrument for casework or database analysis. Typically, this is run by an analyst or technician. However, it may be run by an outside vendor, if it is documented that the performance check happened *after* any/all repairs or adjustments to the instrument.

Performance Check Procedure:

- A standard curve and NTC wells are run according to the procedure in the current version of the Forensic Biology Procedure Manual (FBPM).
- Since the RNase P plate contains these elements, a successful RNase P plate run can also serve as a performance check.

Evaluation:

- This data will be evaluated using the defined quality metrics from the FBPM.

Unacceptable data:

- If any of the quality metrics do not fall in the acceptable range defined in the FBPM, the performance check should be repeated with a new plate.
- If the plate fails a second time, the instrument must be noted as offline, both on the instrument itself and in its logbook, and the Technical Manager must be notified.

Documenting completion and approval/rejection:

- A printout of the report and the reagent worksheet is added to the Maintenance Log binder.
- The PC pass section is completed on the maintenance log, along with the date of completion.

3.8 Applied Biosystems ProFlex Thermal Cyclers (critical instrument)

Reference: [Applied Biosystems ProFlex® PCR System User Guide](#)
[ProFlex PCR System User Guide \(Pub. no. MAN0007697\) \(thermofisher.com\)](#)

3.8.1 Maintenance Procedures

The maintenance log form is in the ProFlex Maintenance Forms document.

Four-week maintenance: During week 3 or 4 of a four-week cycle, the wells and cover should be cleaned, and a self-verification test run. Maintenance is recorded on the maintenance log.

- Before cleaning, power off the instrument by disconnecting the power. Allow the instrument to cool until the heated cover and sample block reach room temperature.
- To clean the sample wells, remove the sample tray from the sample block and set it aside. Use a cotton swab soaked in isopropanol to clean the sample wells thoroughly. Make certain the isopropanol has evaporated completely before reloading a sample tray.
- To clean the heated cover, soak a cotton swab or piece of clean cloth with isopropanol and gently wipe the heated platen. Blot off any remaining isopropanol from the cover and make certain the isopropanol has evaporated completely before restarting the instrument.
- To run a self-verification test:
 - From the Home screen, touch Settings.

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- In the Settings screen, touch Maintenance & Services.
- In the Maintenance & Settings screen, touch Self verification Test.
- In the Self verification test screen, touch Start Test to begin testing. Test takes about ten minutes. Once the test is completed the test results will be displayed in the form of a report.
- Note passing test in Maintenance Log.
 - It is not necessary to save the Self Verification Test report unless the test does not pass.
 - If the Self Verification Test does not pass on two attempts, mark the instrument as offline and notify the DNA Technical Manager to determine a course of action.

3.8.2 Preventive maintenance and other service:

- Annual temperature measurement and verification are performed by a vendor representative. Records are retained and archived annually.
- Annual preventive maintenance (PM) and any service to the instrument, as well as the dates that an instrument is taken out of service or returned to service, are recorded in the maintenance log.

3.8.3 Performance Check

- Following annual preventive maintenance or any service and prior to being used again for casework/database analyses, the instrument must
 - successfully pass a self-verification test
 - successfully pass a performance check, consisting of amplification and analysis of one negative amplification control, plus one positive amplification control in each plate zone (An amplification plate includes six two-column zones: zone one is columns 1 and 2, zone two is columns 3 and 4, and so on). Note: it is NOT necessary to have negative controls in each plate zone; one negative control per plate is sufficient. The performance check may be performed using any one of the amplification kits currently in use at AK SCDL. Controls are evaluated using criteria defined in the Forensic Biology Procedure Manual. Documentation consists of a printout of each of the controls and marking PC following PM/service in the maintenance log. Documentation can demonstrate that each plate zone included one positive control in either one of two ways:
 - Each sample can be named in GMID-X with the well number it occupied on the amp plate (e.g., POS1 – amp well B1)
 - An amplification report can be included with the compiled documentation.

As needed:

- Clean the touchscreen with any commercially available LCD cleaning product, being careful not to scratch the screen.
- If sample wells or heated cover become contaminated, clean thoroughly with a cotton swab soaked in 1:10 solution of Clorox bleach, then rinse with water.

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3.9 Applied Biosystems 3500xl (critical instrument)*References: Applied Biosystems 3500xl Genetic Analyzers User Guide*http://www3.appliedbiosystems.com/cms/groups/mcb_support/documents/generaldocuments/cms_104815.pdf

Annual Preventive Maintenance is performed in-house by manufacturer personnel. The maintenance is recorded on the maintenance log in a binder near the instruments. The service report is also maintained with the instrument records. Additional maintenance, also recorded in the log, is described below. Instrument maintenance records are archived in SharePoint annually.

3.9.1 Maintenance to be performed as needed

- Ensure adequate levels of buffer in reservoirs
- Purge old plate records
 - Click **Library** and select **Plates** in the navigation pane. All plates stored within the library will appear on the screen.
 - Select the plates to be deleted (more than one can be selected at a time).
 - Right click the mouse and select **delete**.

Note: Do not use the purge feature to delete items in the library. Doing so will delete all items with the exception of factory stored items. Thus, all multiplex assays and protocols from other manufacturers will be deleted.

3.9.1.1 Replacing Anode Buffer Container (ABC)

The Anode Buffer Container (ABC) must be replaced after 14 days or 50 injections.

- Allow buffer container to equilibrate to room temperature prior to placing on the instrument.
- Ensure that most of the 1X buffer is in the larger side of the ABC container prior to removing the seal by tilting the container slightly.
- Place the ABC into the Anode end of the instrument, below the pump. (RFID tag will face the instrument).

3.9.1.2 Replacing Cathode Buffer Container (CBC)

The Cathode Buffer Container (CBC) must be replaced after 14 days or 50 injections.

- Allow buffer container to equilibrate to room temperature prior to placing on the instrument.
- Press the tray button on the instrument to bring the autosampler to the forward position.
- Wipe away any condensation on the exterior of the CBC using lint free lab cloth.
- Tilt the CBC back and forth gently to ensure the buffer is evenly distributed and remove the seal.
- Ensure the top of the CBC is dry (failure to do this may result in arcing) and place the appropriate septa on both sides of the CBC.
- Install the CBC on the autosampler.

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3.9.1.3 *Replenishing Polymer*

The polymer must be replaced after 960 samples (or 120 injections) or when it has passed the expiration date.

- Click **Maintenance** (top right of the screen). In the Maintenance Wizards screen, click **Replenish Polymer** (this will take 10 to 20 minutes to complete) and follow the prompts.
- Polymer may be replenished as part of the water wash wizard.

3.9.1.4 *Replacing the Capillary Array*

The capillary is replaced as needed; when indicated by poor data quality.

- The following indications may suggest that a new capillary array is required:
 - Poor sizing precision or allele calling
 - Poor resolution and/or decreased signal intensity
- In the Maintenance Wizards screen click **Install Capillary Array** (this will take 15-45 minutes to complete) and follow the prompts.

Note: Spatial and Spectral Calibrations must be performed anytime an array is replaced. A water wash, water trap flush and performance check must also be completed to verify performance of the array.

3.9.1.5 *Spatial Calibration*

A spatial calibration establishes a relationship between the signal emitted by each capillary and the position where that signal falls on and is detected by the CCD camera. A spatial calibration must be performed when the capillary array has been replaced, the detector door has been opened, or the instrument has been moved. **Note:** A spatial is performed during the Performance Check procedure and does not need to be run separately if the performance check procedure is run.

Performing a Spatial Calibration

- Access the Spatial Calibration screen:
 - Click **Maintenance** and then select **Spatial Calibration** in the navigation pane.
- Under Options, select **NO-Fill** or select **Fill** to fill the array with polymer before starting the calibration.
- Select **Perform QC Checks** to enable the system to check each capillary against the specified range for spacing and intensity.
- Click **Start Calibration**.

Evaluating a Spatial Calibration

- Evaluate the spatial calibration profile to ensure that you see:
 - One sharp peak for each capillary. Small shoulders are acceptable
 - One marker (+) at the top of every peak.
 - Peaks are about the same height.
- If the results meet the above criteria, click Accept Results. If the results do not meet the above criteria, click Reject Results and refer to the Applied Biosystems 3500/3500xl Genetic Analyzer User guide, "Spatial calibration troubleshooting" page 300.
- If the results are acceptable, a printed copy of the passing spatial calibration report is placed in the 3500 Maintenance Logbook. To print a copy of the report, click **View Detail Report**, then click **Print**. In the printer dialog box, select **CutePDF Writer** as the printer. Save this to a thumb drive, which can then be printed from a computer that is networked to a printer.

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3.9.1.6 Spectral Calibration

A spectral calibration creates a de-convolution matrix that compensates for dye overlap.

A spectral calibration should be performed for each chemistry used whenever the capillary array is changed, the CCD camera or laser are realigned or replaced, following preventative maintenance that affects the optical components, or if you see a decrease in spectral separation.

Performing a Spectral Calibration – Dye Set J6 (for use with Global Filer and capillary performance checks) and J6-OSR (for use with Global Filer Express)

- In the Dashboard, Click **Start Pre-heat 60°** at least 30 minutes prior to the start of the run.
- Ensure the consumables are not expired and adequate injections remain.
- Ensure the pump assembly is free of bubbles, run the Remove bubble wizard if needed.
- Thoroughly mix the contents of the DS-36 Matrix Standard (Dye Set J6) tube and spin briefly in a microcentrifuge.
- Prepare the matrix standard by combining the following in a 1.5 mL microcentrifuge tube:
 - For J6 (or J6-OSR) matrix: Standard: 6 µL and Hi-Di Formamide: 294 µL
- Dispense 10 µL of the matrix standard/Hi-Di formamide mixture into the first 24 wells (three columns) of a 96 well CE plate and cover with plate septa.
- Briefly centrifuge the plate containing the standards and verify that each sample does not contain bubbles and is positioned correctly in the bottom of the well.
- Denature at 95°C for 5 minutes. Snap chill for three minutes.
- Place the sample plate into the plate base provided with the instrument.
- Snap the plate cover onto the plate, septa, and plate base.
- Verify that the holes of the plate retainer and the septa are aligned.
- Press the tray button on the instrument to bring the autosampler to the forward position.
- Place the plate in the autosampler with the labels facing you and the notched corner of the plate in the notched corner of the autosampler. Close the instrument doors.
- Access the Spectral Calibration screen:
 - Select **Maintenance**, then click **Spectral Calibration** in the navigation pane.
- Select **96** for the number of wells in the spectral calibration plate and specify the plate location (A or B) in the instrument.
- Select **Matrix Standard** as the chemistry standard and **J6 (or J6-OSR)** as the dye set.
- Select **Allow Borrowing**.
- Click **Start Run**.
- Note: The same spectral plate will need to be run two times: once with J6 and once with J6-OSR.

Performing a Spectral Calibration – Dye Set Promega G5 (for use with PowerPlex Y23)

- In the Dashboard, Click **Start Pre-heat 60°** at least 30 minutes prior to the start of the run.
- Ensure the consumables are not expired and adequate injections remain.
- Ensure the pump assembly is free of bubbles, run the Remove bubble wizard if needed.
- At first use, thaw the 5C Matrix Mix and Matrix Dilution Buffer completely. After first use, store reagents at 2 – 10 C, protected from light.
- Vortex the Matrix Mix for 10 – 15 seconds. Add 10 µL of 5C Matrix Mix to one tube of Matrix Dilution Buffer. Vortex for 10-15 seconds. Note date of dilution on side of tube. Diluted Matrix Mix can be stored up to one week at 2 – 10 C.

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- Add 10 μL of the diluted 5C Matrix Mix to 500 μL of Hi-Di formamide. Vortex for 10-15 seconds.
- Dispense 15 μL of the master mix into the first 24 wells (3 columns) of a 96 well CE plate and cover with a plate septa. **DO NOT HEAT DENATURE.**
- Briefly centrifuge the plate containing the standards and verify that each sample does not contain bubbles and is positioned correctly in the bottom of the well.
- Place the sample plate into the plate base provided with the instrument.
- Snap the plate cover onto the plate, septa, and plate base.
- Verify that the holes of the plate retainer and the septa are aligned.
- Press the tray button on the instrument to bring the autosampler to the forward position.
- Place the plate in the autosampler with the labels facing you and the notched corner of the plate in the notched corner of the autosampler. Close the instrument doors.
- Access the Spectral Calibration screen:
 - Select **Maintenance**, then click **Spectral Calibration** in the navigation pane.
- Select **96** for the number of wells in the spectral calibration plate and specify the plate location (A or B) in the instrument.
- Select **Matrix Standard** as the chemistry standard and **Promega G5** as the dye set.
- Select **Allow Borrowing**.
- Click **Start Run**.

Evaluating a Spectral Calibration

- Passing and failing capillaries are shown in green and red respectively. Borrowed capillaries are shown in yellow with an arrow indicating the adjacent capillary from which results were borrowed. Up to three adjacent-capillary borrowing events are allowed.
- If fewer than the recommended number of capillaries pass, the spectral calibration run will be repeated automatically up to three times.
- View the raw data for each capillary. Ensure that the data meet the following criteria:
 - Order of the peaks in the raw data profile from left to right is orange-red-yellow-green-blue for G5 and Promega G5 Dye Sets, and, orange-red-yellow-green-blue-purple for Dye Set J6.
 - The Quality Value is ≥ 0.95 and the Condition Number is ≤ 8.0 for J6; the Quality Value is ≥ 0.95 and the Condition Number is ≤ 13.5 for G5 and Promega G5.
- If the data for all capillaries meet the above criteria, click **Accept Results**.
- If any capillary data does not meet the criteria click **Reject Results** and refer to the Applied Biosystems 3500/3500xl Genetic Analyzer User guide "Spectral calibration troubleshooting" page 301.
- If the results are acceptable, a printed copy of the passing spectral report is placed in the 3500 Maintenance Logbook. To print a copy of the report, click **View Detail Report**, then click **Print**. In the printer dialog box, select **CutePDF Writer** as the printer. Save this to a thumb drive, which can then be printed from a computer that is networked to a printer.

3.9.2 Daily and Weekly In Use Maintenance

Daily: The computer and instrument are wiped with sterile water on a Kimwipe.

Weekly: The computer and instrument are restarted before the first run of the week.

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3.9.3 Four-week Maintenance

The water wash and water trap flush are performed as part of four-week maintenance and/or anytime an array is replaced. The CBC septa is also replaced as part of the four-week maintenance.

3.9.3.1 *Computer maintenance*

- Defragment the hard drive
Start > Programs > Accessories > System Tools > Disk Defragmenter

3.9.3.2 *Water Wash*

- The water wash may take over 40 minutes to complete
- Click **Maintenance** (top left of screen) on the dashboard.
- Select Wash Pump and Channels to run the wizard. Follow the prompts to completion.

Note: An empty ABC reservoir may be used instead of emptying the reservoir currently on the instrument. Simply remove from the instrument, cover, and set aside. At the completion of the Water Wash Wizard, replace the ABC with the reservoir previously removed from the instrument or a new reservoir.

3.9.3.3 *Water Trap Flush*

- Fill the supplied 20ml Luer lock syringe with warm deionized water. Expel any bubbles from the syringe.
- 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 clockwise.
- Open the Luer fitting by grasping the body of the fitting and turning it counterclockwise approximately one-half turn to loosen.
- Flush 5ml of deionized water through the trap taking extra care not to use excessive force.
- Remove the syringe from the Luer fitting by holding the fitting with one hand while turning the syringe counterclockwise.
- Close the Luer fitting by lightly turning clockwise until the fitting seals against the block.
- Empty the water trap waste container.

3.9.3.4 *Four-week Maintenance for Offline 3500 Instruments*

- Follow the Water Trap Flush procedure described above
- Use a syringe with tubing attached where the polymer would be attached to flush the pump and channels with deionized water
- Ensure that the liquid level in the ABC container is full

3.9.4 Performance Check

A performance check provides for assessment of the instrument system's resolution and its ability to adequately resolve the peaks of an allelic ladder within one base pair. Also, it monitors the ability of the instrument to produce consistent peak heights over the relevant sizing range.

This performance check is performed at a minimum every three months. This performance check is also performed after capillary array changes and after PM or service has been performed on a 3500 instrument prior to resuming use for casework or database analysis.

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Procedure:

- Follow the instructions in the Forensic Biology Casework Procedures Manual to prepare the 3500xl for a run
- If the capillary has been changed, follow the manufacturer's instructions to run a DS-36 (dye set J6) spectral calibration.
- Prepare an allelic ladder master mix by adding the following volumes of reagents to an appropriately sized tube:
 - 12µl GeneScan 600 LIZ
 - 30ul GlobalFiler allelic ladder
 - 288µl Hi-Di Formamide
- Vortex the master mix and spin briefly. Transfer 10µl of the master mix to the appropriate wells (i.e. A1-H3). Briefly centrifuge the plate, then heat and snap chill the plate. Prepare plate assembly and load on the 3500.
- To access the Fragment Install Standard screen: Select **Maintenance**, then select **HID Install Standard** in the navigation pane.
- Select the plate type and plate position in the instrument. Note: you do not create a plate for the performance check – the software uses predetermined positions for the run.
- Click **Start Run**. The run takes about 30 minutes.
- The software evaluates data for all capillaries, including:
 - Nominal allele sizes for all markers
 - Average peak heights
 - Sizing precision
 - Pass/fail

Evaluating results:

- Evaluate the standard data as follows:
 - Examine the number of size standard and allele peaks found for each capillary. The number of expected peaks is shown above the observed number of peaks for each of the capillaries.
 - If the expected number of alleles and size standard peaks are found, click Accept Results.

Unacceptable data:

- If the expected number of alleles and size standard peaks are not found, click Reject results. Rerun the plate. If the check is still not successful, consult the 3500 User Guide for troubleshooting suggestions. If the instrument still does not pass, consult the DNA Technical Manager and notify discipline scientists that the instrument is offline until the issue is resolved. Notification includes marking the instrument itself as offline and adding a note to the maintenance logbook.

Documentation of completion and approval/rejection

- A printed copy of the passing performance check is placed in the 3500 Maintenance Logbook. To print a copy of the report, click **View Detail Report**, then click **Print**. In the printer dialog box, select **CutePDF Writer** as the printer. Save this to a thumb drive, which can then be printed from a computer that is networked to a printer.
 - Since performance checks need to occur every three months, after the completion of the performance check, the next due date will be set for the same day of the month, three months later. For example, if a performance check was completed on February 1, the due date for starting the next performance check would be May 1.

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3.10 Handheld Mechanical Pipettes (critical instrument)

Specifications for Calibrations and Calibration Provider

- Provider must meet ISO 17025 accreditation standards as the time of service
- Scope of accreditation must allow for on-site calibration and must include the range of equipment requiring calibration
- Scheduling requests must be responded to within five days of the request to identify a time and date that is mutually agreed upon by the lab and the calibration provider.
- Calibration services are to be performed on-site, preferably during the same month as the previous year
 - Multiple readings are to be taken at multiple volumes (where applicable) for single channel and multi-channel pipettes.
 - Adjustments to the calibration to be made, as needed.
 - Results to be compared against tolerance agreed upon with the laboratory.
 - Provider must maintain as found and as left measurements, with uncertainty calculations.
 - Calibration data is recorded prior to performing preventive maintenance, resulting in statistical analysis to reflect performance As Found.
 - If a pipette fails As Found, this is noted on the Calibration Certificate for that pipette and will be reported to the laboratory for further instructions on how to proceed with the service.
 - Further data is then recorded after preventive maintenance, and/or any calibration adjustment to report the pipette's performance As Left. If a pipette cannot be repaired and/or fails to meet the required tolerances, it may be tagged as "failed" or "out of service".
 - The Calibration Certificate is then generated including a statistical analysis to reflect on the pipette's overall performance.
 - A certificate must be provided for each pipette which meets all ISO/IEC 17025 (International Standards Organization's standard for Calibration and Test Laboratories) requirements, including uncertainty calculations and real-time environmental conditions.
 - Each certificate shall bear the A2LA Accredited seal with certificate number.
 - The Calibration Certificate must clearly identify if a pipette could not be repaired and/or failed to meet required tolerances.
 - This procedure is driven and designed to meet ISO/IEC 17025 requirements.
 - Labels are required to be attached to each pipette, reflecting the calibration date and next due date.
- Maintenance and repair of pipettes should be provided, as needed.
 - Inspection of internal and external parts performed.
 - Provide general maintenance to sealing system as necessary for restoration of instrument, cleaning, polishing, and lubrication as required.
 - Minor repairs may also be included.

Procedure and evaluation:

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- Pipettes must be bleached before and after calibration.
- Pipettes are calibrated annually in-house by a suitable vendor, using their own procedure and evaluation, for the specified volume range of the pipette. Must meet the above specifications, at a minimum.

Unacceptable data:

- Pipettes identified by the vendor as not passing are labelled as offline and removed from lab workspace.
- Pipettes may be sent to the vendor facility for repair. Pipettes returned from the vendor must pass a performance check, as prescribed by the DNA Technical Manager, prior to being returned to service.

Documentation of completion and approval/rejection:

- ISO certificates of calibration received from the vendor are archived in SharePoint.
- Certificates indicate pipettes as passing or failing.
- Individual pipettes are each labeled with the calibration date and the next due date for calibration.

3.11 Heat block for capillary electrophoresis plate prep (critical instrument)

The heat blocks hold up to two plates. They are always left on and set to ~97°C, and performance is monitored before each use by checking the thermometer in the corresponding side of the heat block.

Performance check

Procedure and evaluation: Prior to putting a plate in the heat block, analyst will check that the thermometer temperature for that side of the heat block is in acceptable range (95 to 99°C).

Unacceptable data: If the thermometer indicates that the heat block side is not within the acceptable temperature range, the analyst will mark the side as offline on the instrument and on the log and notify the Technical Leader.

Documentation of completion and approval/rejection: Analyst will complete the log for each use; a thermometer temperature in range is documentation of a passing result.

3.12 QIAcube® (critical instrument)

3.12.1 QIAcube 1 (QIAcube Classic)

References: QIAcube® User Manual, Version 1.1, 06/2008

3.12.1.1 Regular maintenance procedure

After running a protocol, perform the regular maintenance procedure:

- Wipe down platform with a kimwipe moistened with ethanol and then distilled water.
 - Do not directly spray the inside of the QIAcube with water or ethanol!
- Empty the waste drawer.
 - If necessary, wipe down with a kimwipe moistened with ethanol and then distilled water.

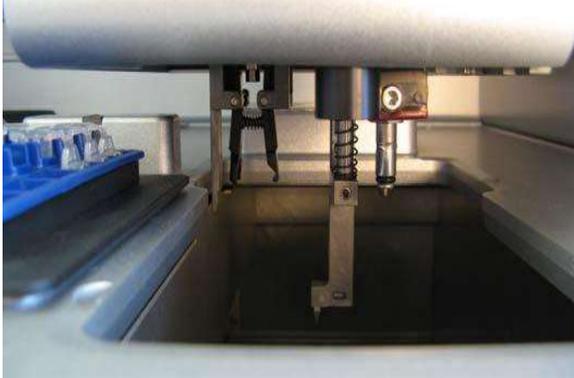
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- Remove used disposable labware and unwanted samples and reagents from the worktable. Discard in biohazardous waste.
 - Plastic rotor adaptors are single use only
- Replace the lids of the reagent bottles and close tightly.
- Rerack tips if there are any partially used racks.

3.12.1.2 Four-week Maintenance Procedure

- Clean the optical sensor, tip adapter, gripper unit (including the gripper), the stabilizing rod, and the spin column lid holder, by wiping these modules with a soft lint-free cloth moistened with water.
 - To gain access to the modules within the robotic arm:
 - Be sure to remove the waste drawer and the labware tray to prevent robotic arm from crashing into tray.
 - “Tools” => “Maintenance” => “Cleaning position”
 - Visually and manually inspect the O-ring to make sure it is intact (not cracked and seated properly).
- 
- Wipe down the following with a kimwipe moistened with ethanol and then distilled water. Wipe dry.
 - Worktable
 - Underneath centrifuge rotor
 - Centrifuge, centrifuge gasket, and centrifuge lid
 - Shaker rack, labware tray, heating adapter, reagent bottle rack, rotor plastic holder
 - Waste drawer liner (and drawer, if needed)
 - Wipe the inside and outside of the QIAcube with distilled water.
 - Do not use alcohol or alcohol-based disinfectants on the QIAcube door.
 - Wipe the touchscreen with a kimwipe moistened with ethanol and then distilled water. Wipe dry with a paper towel.
 - Perform regular maintenance procedure but remove reagent bottles from QIAcube.
 - Perform a Tightness Test
 - The tightness test is performed to check whether the tightness of the pipetting system, including the attached pipetting tip, is sufficient.
 - Load an empty 2 ml microcentrifuge tube in position 1 of the shaker.
 - Fill a reagent bottle with reagent alcohol and place in position 1 of the reagent bottle rack.
 - Load a tip rack of 1000 µl wide-bore filter tips onto the QIAcube.
 - Start Tightness Test
 - In the main menu, press “Tools”.
 - Select “Maintenance”

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- Select “Tightness test”
- Select the appropriate type of filter-tips (“1000 µl wide-bore tips”)
- Press “Start” to start the tightness test with the selected type of filter-tips.
- Follow the instructions displayed in the touchscreen, and press “Start” to start the tightness test.
- 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.
- After the protocol is completed, open the QIAcube door and check if the tube contains liquid.
 - PASS: If the tube is still empty and dry, the tightness of the pipetting system is adequate, and the test result is passing.
 - FAIL: Liquid present in the tube at the end of the test indicates a failure of the test. If you find liquid in the tube, change the O-ring and repeat the test. If the second test fails, the instrument must be taken offline until the issue is resolved. Note the test results in the maintenance documentation and put a note on the instrument to show that it is offline. Notify the technical manager.
- Based on results of tightness test, if necessary, change O-ring (see QIAcube® Tip-Adapter Ring Replacement protocol). NOTE: The ring should be changed at least quarterly. O-ring change is noted in the maintenance log.

3.12.1.3 Semi-annual Maintenance Procedure

- Perform four-week maintenance procedure
- Access to the inside of the centrifuge is required. Lid should be open provided that a protocol is not being run. In case it is closed, to open centrifuge lid:
 - “Tools” => “Maintenance” => “Open lid”
- Switch off the QIAcube at the power switch.
- Remove the buckets from the rotor. Undo the rotor nut on rotor top using the rotor key and lift the rotor off the rotor shaft.
- Rinse the rotor, buckets, and rotor nut in ethanol then distilled water. Use a swab to reach narrow areas. Wipe surfaces dry with a soft lint-free cloth.
- Apply a few drops of mineral oil (Anti-Corrosion Oil (rotor), cat. no. 9018543) on a soft, lint-free cloth or a swab, 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.

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- Clean the inside of the centrifuge, centrifuge gasket, and centrifuge lid with ethanol then distilled water. Wipe dry with lint-free paper towel.
- Check the centrifuge gasket for damage. If the gasket is damaged or shows signs of wear, contact QIAGEN Technical Services.
- Reinstall rotor and buckets
 - The rotor can be mounted in only one orientation. The pin on the rotor shaft fits into a notch on the underside of the rotor directly underneath rotor position
 - Line up position 1 of the rotor with the pin on the rotor shaft and carefully lower the rotor onto the shaft. Install the rotor nut on top of the rotor and tighten using the rotor key supplied with the QIAcube. Make sure that the rotor is securely seated.
 - When replacing the rotor buckets, the side of the rotor bucket that must face toward the rotor shaft is marked with a gray line. Hold the bucket at an angle with the gray line facing the center of the rotor and hang the bucket on the rotor. Check that all buckets are properly suspended and can swing freely.
 - Important: All centrifuge buckets must be mounted before starting a run.

3.12.1.4 *Semi-annual Performance Check*

Approximately once every six months, as well as after repair or service, a performance check will be run on each QIAcube instrument. Note: The PC date performed is the date that the PC was performed in lab (not the review date).

Procedure: The performance check will consist of one known sample (including both sperm and epithelial cells), and one reagent blank sample. Performance check samples will be taken through the casework protocol for differential extraction with automated wash protocol.

Evaluation: Extracts generated in the performance check are quantified, amplified (STR only is sufficient), and analyzed. Resulting data is analyzed according to criteria described in the current version of the Forensic Biology Procedure Manual. In addition, they are checked to make sure the correct profiles were obtained. A passing performance check consists of correct and complete profiles for both

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the sperm and epithelial fractions of the positive control sample, plus amplified reagent blank profiles without extraneous DNA.

Note: For this evaluation, reagent blanks must be dried down and fully amplified if the quantification result indicates any possible DNA present. If quantification results do not indicate possible DNA (i.e., Undetermined or 0.0000) then the blank extracts can be amplified without being dried down and rehydrated.

Unacceptable data: A performance check failure would be an incomplete or incorrect positive control profile, or a reagent blank profile with detected DNA. However, an incomplete profile, and some instances of reagent blank contamination, may be indicative of an issue at the amplification stage. Such samples should be re-amplified. If re-amplification does not yield a full, correct profile for a positive control sample and/or a reagent blank profile without extraneous DNA, then the performance check fails. The instrument must be taken offline and the Technical Manager must be notified, so that a further course of action can be determined by the Technical Manager.

Documentation of completion and approval/rejection: Performance check documentation includes electropherograms of the successful positive and negative controls. If the performance check was performed as a part of a casework central log, the central log may be referenced. If the performance check was performed alone, documentation such as allelic ladders, amplification controls, etc. should be included with the performance check paperwork. An electronic scan of the compiled performance checks documentation for a calendar year is then stored on the laboratory network at the end of the year.

3.12.2 QIAcube® 2 (critical instrument)

References: QIAcube® Connect User Manual, January 2022

3.12.2.1 Regular maintenance procedure

After running a protocol, perform the regular maintenance procedure:

- Wipe down platform with a kimwipe moistened with ethanol and then distilled water.
- Do not directly spray the inside of the QIAcube with water or ethanol
- Empty the waste drawer.
- If necessary, wipe down with a kimwipe moistened with ethanol and then distilled water.
- Remove used disposable labware and unwanted samples and reagents from the worktable. Discard in biohazardous waste.
- Plastic rotor adaptors are single use only
- Replace the lids of the reagent bottles and close tightly.
- Rerack tips if there are any partially used racks. • Following the last run of the day, perform a UV run. Under Tools, select the UV Run tab. Select two cycles (1 cycle = 12 minutes) and Start.

3.12.2.2 Four-week Maintenance Procedure

- Perform the daily in use maintenance procedure before performing the four-week maintenance.
- On the touchscreen, press the Tools icon on the menu bar, then press the Maintenance tab and the Monthly subtab. This will bring up a walk-through for the four-week maintenance. Note: As a part of four-week maintenance, inspect all parts that are cleaned and notify the Technical Manager if any broken parts are observed.

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- Close the hood.
- Clean the worktable with a Kimwipe moistened with ethanol, followed by a Kimwipe moistened with distilled water. Wipe dry afterwards. Note: do not use alcohol to clean the hood.
- Clean the touchscreen with a Kimwipe moistened with ethanol and wipe dry afterwards. Note: Take care that no liquid runs down the touchscreen.
- Clean the outer hood with a Kimwipe moistened with distilled water.
- Clean the shaker adaptor (grey), shaker tray (metal), shaker rack plugs, and buffer bottle rack with a Kimwipe moistened with ethanol, followed by a Kimwipe moistened with distilled water; wipe dry afterwards.
- Cleaning the robotic arm
 - Press the Tools icon, then the Maintenance tab and the Robotic arm subtab. Instructions are listed on the screen and described below.
 - Make sure used labware, adaptors, and reagents are removed from the worktable. Close the hood.
 - Press Next to move to cleaning position.
 - Open the waste drawer and carefully clean the optical sensor, tip adaptor, gripper unit (including the gripper), the rotor adaptor stabilizing rod, and the spin column lid holder, by wiping these modules with a soft lint-free cloth moistened with water. Wipe these items dry.
 - Visually and manually inspect the O-ring to make sure it is intact (not cracked and seated properly).
- Close the hood and press Done.
- Perform a Tightness test. The tightness test is performed to check whether the tightness of the pipetting system, including the attached pipetting tip, is sufficient. Note: in addition to four-week maintenance, this test must be performed after replacing the tip adaptor O-ring.
- Press the Tools icon, then the Maintenance tab, then the Tightness subtab. The instructions provided on the screen are summarized in the steps below.
 - Open the hood and load a 1000 μ L tip rack with at least one 1000 μ L narrow bore tip in the tip rack position 1 Note: Narrow bore tips are **only** used for this test.
 - Load an empty 2 ml microcentrifuge tube in position 1 of the shaker.
 - Fill a reagent bottle with reagent alcohol and place in position 1 of the reagent bottle rack.
 - Close the hood and press Next. 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 discarded into the waste afterwards.
 - Wait until the test has been completed and press Next.
 - After the protocol is completed, open the QIACube door and check if the tube contains liquid.
 - PASS: If the tube is still empty and dry, the tightness of the pipetting system is adequate, and the test result is passing.
 - FAIL: Liquid present in the tube at the end of the test indicates a failure of the test. If you find liquid in the tube, change the O-ring and repeat the test. If the second test fails, the instrument must be taken offline until the issue is resolved. Note the test results in the maintenance documentation and put a note on the instrument to show that it is offline. Notify the technical manager.
- Based on results of tightness test, if necessary, change O-ring and repeat tightness test. For directions on O-ring replacement, press the Tools icon on the menu bar, then press the Maintenance tab and the O-ring subtab. NOTE: The ring should be changed at least quarterly, using the special tool designated specifically for Qiacube Connects. O-ring change is noted in the maintenance log.

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3.12.2.3 Semi-annual Maintenance Procedure

- Perform four-week maintenance procedure
- Clean the centrifuge
- Press the Tools icon, then press the maintenance tab and the Centrifuge subtab. Press Start. Follow the directions on the screen, which are summarized in the steps below.
- The centrifuge lid must be open to allow access to the inside of the centrifuge. If the lid does not open automatically, close the hood and press the Open Centrifuge Lid button.
- Switch off the QIAcube at the power switch and perform the following cleaning tasks.
 - Cleaning the rotor and buckets
 - Cleaning the centrifuge
 - Maintenance of the rotor nut
 - Installing the centrifuge rotor and buckets
- When cleaning is completed, turn on the instrument and log in. perform the centrifuge operation check.
- Cleaning the rotor and buckets • Remove the buckets from the rotor. Undo the rotor nut on rotor top using the rotor key and lift the rotor off the rotor shaft.



- This is a rotor key:
- Rinse the rotor, buckets, and rotor nut in ethanol then distilled water. Use a swab to reach narrow areas. Wipe surfaces dry with a soft lint-free cloth or use canned air.
- Apply a few drops of mineral oil (Anti-Corrosion Oil (rotor), cat. no. 9018543) 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.

Cleaning the centrifuge

- Clean the inside of the centrifuge, centrifuge gasket, and centrifuge lid with ethanol then distilled water. Wipe dry with lint-free paper towel.
- Check the centrifuge gasket for damage. If the gasket is damaged or shows signs of wear, contact QIAGEN Technical Services.



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Maintenance of the rotor nut:



- After cleaning the rotor thread, apply a few drops of mineral oil (Anti-Corrosion Oil (rotor), cat. no. 9018543) on a soft, lint-free cloth, and wipe the thread. A thin, invisible oil film should cover the bucket mount and rotor claw, but no droplets or smear should be apparent.

Installing the centrifuge rotor and buckets

- The rotor can be mounted in only one orientation. The pin on the rotor shaft fits into a notch on the underside of the rotor directly underneath rotor position 1. Line up position 1 of the rotor with the pin on the rotor shaft and carefully lower the rotor onto the shaft.
- Install the rotor nut on top of the rotor and tighten using the rotor key supplied with the QIAcube. Make sure that the rotor is securely seated.
- When replacing the rotor buckets, the side of the rotor bucket that must face toward the rotor shaft is marked with a gray line. Hold the bucket at an angle with the gray line facing the center of the rotor and hang the bucket on the rotor. Check that all buckets are properly suspended and can swing freely. Important: All centrifuge buckets must be mounted before starting a run.

Centrifuge operations check following cleaning:

- This check is performed to ensure that no residual plastic parts are still in the centrifuge.
- Switch the instrument on and log in.
- Press the Tools icon and then the Run Modules tab.
- In the Set speed and Set duration fields, set the speed to 10,000 g and the duration to one minute, respectively.
- Press Start to begin the centrifuge run. Carefully listen to the sound during centrifugation. • If any grinding, rattling, or crunching sounds are heard during centrifugation, repeat the cleaning procedures.
- If no unusual sound can be heard, the check is considered complete.

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3.12.2.4 *Semi-annual Performance Check*

Approximately once every six months, as well as after repair or service, a performance check will be run on each QIAcube instrument.

Procedure: The performance check will consist of one known sample (including both sperm and epithelial cells), and one reagent blank sample. Performance check samples will be taken through the casework protocol for differential extraction with automated wash protocol.

Evaluation: Extracts generated in the performance check are quantified, amplified, and analyzed (STR only is sufficient). Resulting data is analyzed according to criteria described in the current version of the Forensic Biology Procedure Manual. In addition, they are checked to make sure the correct profiles were obtained. A passing performance check consists of correct and complete profiles for both the sperm and epithelial fractions of the positive control sample, plus amplified reagent blank profiles without extraneous DNA.

Note: For this evaluation, reagent blanks must be dried down and fully amplified if the quantification result indicates any possible DNA present. If quantification results do not indicate possible DNA (i.e., Undetermined or 0.0000) then the blank extracts can be amplified without being dried down and rehydrated.

Unacceptable data: A performance check failure would be an incomplete or incorrect positive control profile, or a reagent blank profile with detected DNA. However, an incomplete profile, and some instances of reagent blank contamination, may be indicative of an issue at the amplification stage. Such samples should be re-amplified. If re-amplification does not yield a full, correct profile for a positive control sample and/or a reagent blank profile without extraneous DNA, then the performance check fails. The instrument must be taken offline, and the Technical Manager must be notified, so that a further course of action can be determined by the Technical Manager.

Documentation of completion and approval/rejection: Performance check documentation includes electropherograms of the successful positive and negative controls. If the performance check was performed as a part of batched casework, the relevant batch documentation may be referenced. If the performance check was performed alone, documentation such as allelic ladders, amplification controls, etc. should be included with the performance check paperwork. An electronic scan of the compiled performance checks documentation for a calendar year is then stored on the laboratory network at the end of the year

3.13 RapidHIT ID System (critical instrument)

Reference: RapidHIT ID System Guide v1.3.1 User Guide

Maintenance Procedures

3.13.1 Routine maintenance as needed:

- Clean the touchscreen by powering off the internal computer (the button on the FRONT on the instrument), spraying with a non-abrasive glass cleaner, and gently wiping the screen with a lint-free cloth or lab tissue.
- Replace the primary cartridge.
 - Refer to the User Guide for directions on changing the primary cartridge.

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- Once the new primary cartridge is installed, complete a Rapid Primary Cartridge Change form to document successful completion of all required controls and assign a lot number and expiration date

3.13.2 Weekly:

- To maintain appropriate buffer levels and conditions in the primary cartridge, the instrument needs to be run at least once every calendar week (exact time interval may depend on relative humidity and environmental conditions). Note that the required time interval is once per calendar week; it is acceptable for the interval between run date to be more than seven days provided the maintenance is addressed once per calendar week. This can be accomplished by running a sample cartridge, or by leaving a utility cartridge or empty previously used sample cartridge in the instrument during a prolonged period of disuse. At least once per week, the instrument must be checked to see if action is required. If an instrument is left idle for more than one week, meaning no samples were run AND no utility/empty cartridge was left in the instrument, a performance check is required before the instrument can be used for database or casework analysis.

3.13.3 Semi-annually:

- Transfer all run data to SharePoint for storage.
 - Note: This must be performed while logged in as an Admin
 - Insert a USB drive into the USB port on the front of the instrument. The Removable Drives field displays the name of the USB device.
 - In the settings screen, touch Start Backup. When the procedure is complete, a message is displayed.
 - Zip the file and store in the designated location in SharePoint.
 - Add a note to the comment section of the maintenance log along with date and initials – Semi-annual data backup performed.

3.13.4 Performance Check

At least annually, following an idle period, or following service/repairs, a performance check will be run prior to using the instrument for casework or database analysis. Typically, this is run by an analyst or technician. However, it may be run by an outside vendor, if it is documented that the performance check happened *after* any/all repairs or adjustments to the instrument.

Procedure:

- Analyze a buccal swab from a previously typed staff member

Evaluation:

- This data will be evaluated using the defined quality metrics from the FBPM.

Unacceptable data:

- If any of the quality metrics do not fall in the acceptable range defined in the FBPM, the performance check should be repeated with a new buccal swab.
- If the sample fails a second time, the instrument must be noted as offline, both on the instrument itself and in its logbook, and the Technical Leader must be notified.

Documenting completion and approval/rejection:

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- Electropherogram for the passing sample is printed and retained in the instrument maintenance log until annual archiving

PC = Pass is noted in the comments section on the maintenance log, along with the date of completion.

3.14 QIAGEN TissueLyser III

References: HB-3241-001_UM_TissueLyserIII_user manual

3.14.1 Regular Maintenance (After Each Use)

- **Instrument**
 - Power off instrument
 - Clean workable surfaces using a Kim wipe moistened with 70% ethanol
 - Immediately follow up with a Kim wipe moistened with distilled water
 - Do NOT directly spray the ethanol or distilled water inside/on the instrument!
 - Do NOT use bleach, as it may react with the buffer reagents.
 - Check the clamp for any damage
- **Grinding Jar Set**
 - First, clean each component with mild soap and water using a single-use soft brush. Dry with a Kimwipe or similar.
 - Try not to damage the surface of the jar or ball.
 - Next, squirt DNA Away on a Kimwipe and clean each component. Dry with a Kimwipe or similar.
 - Fill the sonicator with clean water. Sonicate grinding jar set pieces for 5-10 minutes.
 - Then, use a Kimwipe moistened with ethanol to clean the jar and ball. Dry with a Kimwipe or similar.
 - Finally, rinse with distilled water, then dry components Dry with a Kimwipe or similar.
 - Check for any wear or damage. Place the larger part of the jar upside down on the drying rack. The smaller part of the jar and balls can be stored on the shelf at the bottom of the rack.

3.14.2 Six-Month Regular Safety Check

- Install both Grinding Jar Sets in the instrument
- Switch on the instrument
- Set the frequency to 30 Hz
- Press "START"
- Raise the hood.
 - Note: The motor should switch itself off and "E50 SECURITY CIRCUIT" should appear on the screen.
- Lower the hood.
 - Note: The motor should NOT switch itself on again. If there are any defects, contact QIAGEN Technical Services to request repair of the instrument.

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Appendix A: Revision History

Location	Revision made
throughout	Grammar, spelling corrections, formatting updates. Updated references to 7500 to QuantStudio 5
Section 1.2	Added (*) and comment to indicate reagents that are entered into LIMS-DNA by the person verifying (GF/PPY23) or rehydrating (GTD)
Section 1.3	Removed KM solutions A, B, and C.
Section 1.4	Updated 1 st bullet and 2 nd to last bullet in section. Updated name of ABACard p30 test. Updated name of ABACard HemaTrace. Added Phenolphthalein Presumptive Blood Test Kit.
Section 2.1	Updated floor cleaning to every two months.
Section 2.2	Added paragraph on performance checks following seismic activity.
Section 3.5.2	Updated O-ring(s) to D-ring(s) throughout section.
Section 3.7	Updated maintenance plan for QuantStudio 5 instruments
Section 3.8.3	Under performance check procedure, changed "An amplification worksheet" to "An amplification report".
Section 3.14.1	Updated cleaning instructions for grinding jar set.
Appendix B	Updated performance check plan for QuantStudio 5 instruments

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Appendix B: Performance Check / Calibration Program Summary

The checks summarized in this table are in addition to regular maintenance, performance checks for new equipment and performance checks required following service or preventive maintenance performed by an outside vendor.

Instrument	Calibration, Performance Check (PC) or Preventive Maintenance (PM)	Frequency	Performed by
Immersion thermometers	PC	Semi-annual	Lab technician (or analyst)
Analytical Balance	PC	Quarterly	Lab technician (or analyst)
Reference Mass Set	Calibration	Annual	Outside vendor
EZ1 Advanced-XL	PC	Semi-annual	Lab technician (or analyst)
EZ2 Connect	PM	Annual	Outside vendor
Thermomixers	PC	Semi-annual	Lab technician (or analyst)
Quant Studio 5 RT-PCR Instruments	PM/PC	Annual	Outside vendor (PC performed by lab technician if not included at end of PM)
ProFlex Thermal Cyclers	PM	Annual	Outside vendor
3500 Genetic analyzers	PC	Every three months and after each capillary change	Lab technician (or analyst)
3500 Genetic analyzers	PM	Annual	Outside vendor
Handheld mechanical pipettes	Calibration	Annual	Outside vendor
Post-PCR plate prep heat block	PC	Prior to each use	Lab technician or analyst
QIAcube Classic	PC	Semi-annual	Lab technician (or analyst)
QIAcube Connect	PC	Semi-annual	Lab technician (or analyst)
RapidHIT Instrument	PC	Annual	Lab technician (or analyst)