Procedure for DNA Extraction

1.0 Purpose – This procedure specifies the steps for performing DNA extractions using the EZ1 Advanced XL BioRobot, the EZ2 Connect Fx BioRobot, and the QIAsymphony SP BioRobot.

2.0 Scope – This procedure applied to Forensic Scientists in the Forensic Biology Section who perform DNA extractions for forensic casework.

3.0 Definitions – See Section Definition List

4.0 Equipment, Materials, and Reagents

- Diluted Buffer ATL
- Buffer ATL
- Buffer MTL
- Tris/EDTA Solution (TE)
- Dithiothreitol (DTT)
- BTmix
- Rinse/lysis solution
- Calibrated pipets (various sizes)
- ART Pipet Tips (or equivalent, various sizes)
- Qiagen EZ1 Advanced XL BioRobot
- Qiagen EZ2 Connect Fx BioRobot
- Qiagen QIAsymphony SP BioRobot
- Qiagen EZ1&EZ2 DNA Investigator Kit (reagent cartridge, ProK, carrier RNA)
- Qiagen QIAsymphony DNA Investigator Kit (Buffer ATL, Buffer ATE, reagent cartridge, ProK, carrier RNA)
- Qiagen sample prep cartridges
- Qiagen 8-rod covers
- Thermomixer
- Autoclaved microcentrifuge tubes (various sizes)
- Autoclaved Spin Ease Baskets (or equivalent)
- 2mL tubes with a lyse & spin basket (or equivalent)
- Sterile tubes (multiple sizes)
- Vortex Mixer
- Various lab equipment (various disposable conical tubes, lab tape, lab coat, lab gloves, microcentrifuge tubes and rack, wipes, etc)
- 10% Bleach solution
- Isopropyl Alcohol
- Decon-quat

5.0 Procedure

5.1 Overview

5.1.1 All known samples shall be extracted separately from unknown samples. Thermomixers shall be designated exclusively for either "knowns" or "unknowns."

5.1.2 For casework unknowns, a portion of each swab present shall be cut for analysis. The amount of sample taken shall be determined based upon the scientist's evaluation of the sample (e.g., amount of staining, sperm quantity recorded).

5.1.3 Make a master mix containing the pre-processing reagents using the volumes list for the sample type (allowing for 1-2 extra samples for pipetting variation). Reagents will be added to the sample tubes from this master mix and not from the stock or aliquot bottles.

5.1.4 If a batch of samples being extracted is in excess of 30 total samples (knowns and unknowns) then witnessing steps shall be added when multiple tubes are open simultaneously during the transfer of tubes (e.g., Qiagility setup). This witnessing will be documented in the case notes. Any additional exemptions to extraction batch processing shall be approved only with written documentation from the Technical Leader.

5.1.5 Negative extraction control

5.1.5.1 For each case, a reagent blank shall be prepared each time an extraction set is begun (i.e., knowns and unknowns). This blank will consist of the reagents used in the extraction process and shall be treated the same as other samples throughout the entire process. Also, the final volume of this control shall be the same as the forensic sample(s) brought up in the most minimal volume and amplified using the maximum volume.

Note: If hair roots are processed within an extraction set, a separate reagent blank shall be prepared.

5.1.5.2 Due to the amount of sample needed for an extraction (i.e., number of swabs, size of the cutting) it may be necessary to split the extraction of a sample across multiple extraction tubes. In such instances, multiple reagent blanks will need to be created. At the point when the separate extractions of the sample are recombined, prior to amplification, the same number of reagent blanks must also be combined. This ensures that the same quantity of reagents have been processed in the reagent blank as the given sample.

5.1.5.3 It is acceptable to run more than one reagent blank in anticipation of having to re-run or dilute samples for amplification.

5.1.5.4 If additional extractions are performed, the associated negative extraction controls shall have a unique identifier (different date will suffice as identifier).

5.1.5.5 The requirement for extracting a reagent blank concurrently with each case applies to samples extracted on or after July 1, 2009. If the sample is resubmitted for additional analysis and the extraction occurred prior to this date, there may not be reagent blanks present to reprocess. A note will be added to the case notes that details this exception. A blank consisting of the same amp grade water being used in the sample(s) will be prepared and processed for these samples. Cases processed after this date shall require reagent blanks for any reprocessing requests to proceed.

5.1.5.6 Reagent blanks must be processed concurrently with the associated samples, not consecutively. Extractions must be occurring at the same time to be considered concurrent. For example, runs occurring at the same time on multiple EZ1 or EZ2 extraction robots would be considered concurrent while consecutive runs on the same extraction robot would not be concurrent. Robots must be the same model and be using the same program for the runs to be considered a concurrent set. QIAsymphony runs processed as one batch may be divided between the four sample carriers to account for different protocol runs and still be concurrent runs.

5.1.6 All tubes shall be labeled with a unique identifier.

5.1.7 If Sexual Assault Evidence Collection Kit samples are submitted for analysis, samples must be processed using differential extraction unless the analyst determines from additional information present that it is not required (e.g., clearly indicated as digital only). Samples processed prior to direct-to-DNA processing techniques (previously tested for body fluid only) shall undergo differential extraction. If all evidence was not previously tested (e.g., partially tested kit) those samples should be extracted. This does not apply to samples and controls being processed using Promega Casework Direct Kit.

5.1.8 Instruments and protocols have been validated for use for the following sample types:

5.1.8.1 EZ1 Advanced XL: Knowns, Unknowns, Hairs, Shell Casings, Samples with identified/possible sperm, Bones/Teeth

5.1.8.2 EZ2 Connect Fx: Knowns, Unknowns, Hairs, Shell Casings, Samples with identified/possible sperm, Bones/Teeth

5.1.8.3 QIAsymphony SP: Known, Unknowns, Hairs, Samples with identified/possible sperm

5.2 Preprocessing of Known and Unknown Samples

5.2.1 Aseptically place the sample into a labeled 1.5 mL microcentrifuge tube or a lyse &spin basket with a 2 mL tube (or equivalent).

5.2.2 To the sample, add the following pre-processing reagents, depending on the instrument being used for DNA purification:

5.2.2.1 EZ1 Advanced XL or EZ2 Connect Fx:

475 µL diluted Buffer ATL
25 µL Proteinase K
1 µL carrier RNA

5.2.2.1.1 Aseptically pipette 501 µL from the master mix into each sample tube. Vortex briefly.

5.2.2.2 QIAsymphony SP:

475 µL Buffer ATL
25 µL Proteinase K

5.2.2.2.1 Aseptically pipette 500 µL from the master mix into each sample tube. Vortex briefly.

5.2.3 Incubate the samples for approximately 15 minutes to 2 hours (based upon the type of sample and not to extend overnight) at 56 °C in a thermomixer set to approximately 700 rpm. If samples cannot be extracted immediately after incubation, then place in refrigerator and heat back to 56 °C before proceeding.

5.2.4 If using a 2 mL tube with a lyse & spin basket (or equivalent), spin in a microcentrifuge at high speed for at least 30 seconds to activate the basket and force the extraction fluid into the tube. If any liquid remains in the basket, repeat spin. Remove the basket and discard it into a biohazard waste container.

5.2.5 If using a 1.5 mL tube, spin briefly in a microcentrifuge to force condensate into the bottom of the tube. Aseptically transfer the sample(s) into a basket insert. Place the basket back in the tube containing the stain extract and cap the tube. Spin in a microcentrifuge at high speed for at least 30 seconds, repeating if liquid remains in the basket. Remove the basket and discard it into a biohazard waste container.

5.2.6 For samples being placed on the EZ1 Advanced XL BioRobot "Large Volume" protocol for DNA purification, add 400 µL warm buffer MTL to the sample tube. This step is not needed for samples being processed on the "Trace" protocol on the EZ1.

5.2.7 Proceed to **5.6** (EZ1 Advanced XL), **5.7** (EZ2 Connect Fx), **or 5.8** (QIAsymphony SP), depending on instrument being used, to continue with DNA purification.

5.3 Preprocessing of Hair Roots

5.3.1 Hair roots are cut and placed in either a 1.5 mL tube or a 2 mL screw cap tube by the Trace Evidence Section and transferred to the Forensic Biology Section.

5.3.2 To the sample, add the following pre-processing reagents, depending on the instrument being used for DNA purification:

5.3.2.1 EZ1 Advanced XL or EZ2 Connect Fx:

160 µL diluted Buffer ATL
20 µL Proteinase K
20 µL DTT
1 µL carrier RNA

5.3.2.1.1 Aseptically pipette 201 µL from the master mix into each sample tube.

5.3.2.2 QIAsymphony SP:

160 µL Buffer ATL
20 µL Proteinase K
20 µL DTT

5.3.2.2.1 Aseptically pipette 200 µL from the master mix into each sample tube.

5.3.3 Vortex briefly on low speed and spin briefly in a microcentrifuge to force the cutting into the extraction fluid.

5.3.4 Incubate the samples for approximately 1 hour at 56 °C in a thermomixer. If samples cannot be extracted immediately after incubation, then place in refrigerator and heat back to 56 °C before proceeding.

5.3.5 Spin briefly in a microcentrifuge to force condensation into the bottom of the tube.

5.3.6 Proceed to **5.6** (EZ1 Advanced XL), **5.7** (EZ2 Connect Fx), **or 5.8** (QIAsymphony SP), depending on the instrument being used, to continue with DNA purification.

5.4 Preprocessing of Samples with Identified/Possible Sperm

5.4.1 Aseptically place the sample into a labeled microcentrifuge tube.

5.4.2 To the sample, add the following pre-processing reagents, depending on the instrument being used for DNA purification:

5.4.2.1 EZ1 Advanced XL or EZ2 Connect Fx:

475 µL diluted Buffer ATL
25 µL Proteinase K
1 µL carrier RNA

5.4.2.1.1 Aseptically pipette 501 µL from the master mix into each sample tube.

5.4.2.2 QIAsymphony SP:

475 µL Buffer ATL
25 µL Proteinase K

5.4.2.2.1 Aseptically pipette 500 µL from the master mix into each sample tube.

5.4.3 Incubate the samples for approximately 1 hour, not to extend overnight at 56 °C in a thermomixer set to approximately 700 rpm. If samples cannot be extracted immediately after incubation, then place in refrigerator and heat back to 56 °C before proceeding.

5.4.4 Spin briefly in a microcentrifuge to force condensate into the bottom of the tube. Aseptically transfer the material to a basket insert. Place the basket insert into the tube containing the stain extract. Spin in a microcentrifuge at high speed for 30 seconds and make sure all the liquid has transferred out of the catch basket. Remove the basket and discard it into a biohazard waste container.

5.4.5 QIAcube Instrument

5.4.5.1 Label the adaptor, 1.5 mL microcentrifuge tube, and 2 mL flat bottom tube with at least the QIACube centrifuge bucket number, which corresponds with the DNA workbook. Secure the 1.5 mL microcentrifuge tube and lid into the adaptor and make sure the tube and lid are flush.

5.4.5.2 While pipetting up and down to dislodge any pellet which may have formed, transfer the liquid to its clean corresponding 1.5 mL microcentrifuge tube within the adaptor.

5.4.5.3 Turn on the QIACube; ensure the DiH2O bottles are filled, lids are off, and placed in both well 1 and well 2 in the reagent bottle rack; and the proper tips are filled.

5.4.5.4 Place the 2 mL flat bottom tubes, without lids into the shaker which corresponds to the DNA workbook set up. Place the rubber stopper (or tape can be used) to cover the shaker slots. The 2 mL flat bottom tubes will be reported as Fraction 1 (formerly the non-sperm fraction).

5.4.5.5 Add the adaptors to the corresponding buckets in QIACube centrifuge. Double check to make sure all tubes correspond with the workbook and bucket positions, all lids are off, adaptors are flush in the buckets, and tips are full. Close the QIACube lid.

Note: Once the lid is closed and a program has started DO NOT Open the lid. This will cause the program to stop and will have to be re-started back to the beginning. If the program is still running, note the message code and report it to the DNA technical leader for further direction.

5.4.5.6 Select the 12A program. Follow touch screen prompts until the selected program starts. When the program stops, remove the 2 mL flat bottom tubes and place in a rack.

5.4.5.7 Once Fraction 1 (formerly non-sperm fraction) has been removed, proceed to **5.6** (EZ1 Advanced XL), **5.7** (EZ2 Connect Fx), **or 5.8** (QIAsymphony), depending on the instrument being used, to continue with DNA purification.

5.4.5.7.1 For samples being placed on the EZ1 Advanced XL BioRobot for DNA purification, add 400 µL warm buffer MTL to each Fraction 1 sample tube.

5.4.5.8 The samples located within the 1.5 mL microcentrifuge tube, within the adaptors, are considered Fraction 2 (formerly the sperm fraction). Aseptically remove the adaptors one at a time. Aseptically remove the 1.5 mL microcentrifuge tube from each adaptor, place in a tube rack, and discard the adaptor into a biohazard waste container.

5.4.5.9 Prepare a slide for microscopic examination. Slides will be made only for items that did not have an existing smear/slide.

5.4.5.9.1 Label slides and slide mailer with the case number and item number. Mark a circle on the slide with a wax pencil where the sample will be added.

5.4.5.9.2 Pipette up and down to disturb the pellet and add 5µL of the liquid to the corresponding slide.

5.4.5.9.3 Place on a hot plate until dry and place it in the labeled slide mailer. Do not package slides from multiple cases in the slide mailer.

5.4.5.10 To the sample, add the following reagents, depending on the instrument being used for DNA purification.

5.4.5.10.1 EZ1 Advanced XL or EZ2 Connect Fx:

160 µL diluted Buffer ATL
10 µL Proteinase K
40 µL DTT
1 µL carrier RNA

5.4.5.10.1.1 Aseptically pipette 211 µL from the master mix into each Fraction 2 tube, secure the lids, and vortex.

5.4.5.10.2 QIAsymphony SP:

160 µL Buffer ATL
10 µL Proteinase K
40 µL DTT

5.4.5.10.2.1 Aseptically pipette 210 µL from the master mix into each Fraction 2 tube, secure the lids, and vortex

5.4.5.11 Incubate the samples for 10 minutes at 70 °C in a thermomixer set to approximately 700 rpm.

5.4.5.12 Spin the sample tube in a microcentrifuge to force all the fluid to the bottom of the tube. DNA from fraction 2 can now be purified from this tube.

5.4.5.13 Proceed to **5.6** (EZ1 Advanced XL), **5.7** (EZ2 Connect Fx), or **5.8** (QIAsymphony SP), depending on the instrument being, to continue with DNA purification.

5.5 Preprocessing of Fired Shell Casings

5.5.1 Prepare rinse/lysis solution master mix by combining 500 µl of diluted buffer ATL and 30 µl of BTmix (2 mg/mL BSA and 62.5 mg/mL GGH tripeptide) per sample. Analysts may increase sample count by 1-2 samples to ensure sufficient volume. Gently mix solution.

5.5.1.1 Preparation of BTmix to a final concentration contains 2 mg/mL BSA and 62.5 mg/mL GGH tripeptide. To prepare GGH precursor solution: Add 743 µL nuclease free water to 100 mg GGH tripeptide (~800 µL final solution volume.) Add an equivalent volume of 4 mg/ml BSA precursor (64 µL of 50 mg/mL BSA to 736 µL nuclease free water.) Combine to create ~1.6 mL working BTmix. Aliquot BTmix into tubes and freeze until use. Aliquots may be stored for 6 months in freezer.

5.5.2 Hold the cartridge case with the head stamp down over an empty collection tube/beaker. Up to 3 casings may be collected together, based on the evidence and relevant case information.

5.5.3 Rinse the cartridge with 500 µL of the rinse/lysis solution.

5.5.4 Repeat the rinse 2-4 times, turning the casing during and after each rinse to ensure the entire surface is covered. The rinse/lysis solution is re-used for each rinse. Use ~400 µL of the rinse/lysis solution for subsequent rinses (to reduce foam formation during the process). Ensure head stamp area is included in the rinse process.

5.5.5 Swab all outer surfaces of the casing with one sterile foam swab. If multiple cartridge casings are processed together, use the same swab. Place the swab head in the Lyse & Spin basket (or equivalent) for extraction.

5.5.6 Repeat rinse process using ~400 µL of the rinse/lysis solution as described in 5.5.4.

5.5.7 Swab all outer surfaces of the casing with one sterile cotton swab. If multiple casings are processed together, use the same swab. Place the swab head in the Lyse & Spin basket for extraction (same basket as **5.5.5**).

5.5.8 Rinse cartridge casings with 70% ethanol for approximately 5 seconds and air dry or wipe with Kimwipe (or equivalent) prior to repackaging.

5.5.9 Transfer entire volume of rinse/lysis solution to Lyse & Spin basket (or equivalent).

5.5.10 Add 25 µL of Proteinase K for DNA extraction and 1 µL cRNA.

5.5.11 Incubate the samples for ~2 hours at 56 °C in a thermomixer set to ~700 rpm.

5.5.12 Spin in a microcentrifuge at high speed for at least 30 seconds to activate the spin basket and force the extraction fluid into the tube. Repeat spin if liquid remains.

5.5.12.1 For samples being placed on the EZ1 Advanced XL BioRobot for DNA purification, add 400 µL warm buffer MTL to each sample tube.

5.5.13 Proceed to **5.6** (EZ1 Advanced XL) or **5.7** (EZ2 Connect Fx), depending on the instrument being used, to continue with DNA purification. Samples can be eluted to a final volume of 40 µL or 50 µL (dependent upon if other sample types are being processed concurrently on the EZ1 instrument) or 20 µL or 50 µL (dependent upon if other sample types are being processed concurrently on the EZ2 instrument).

5.5.14 Upon completion of DNA purification on the EZ1 or EZ2 instrument, concentrate the sample(s) to a final volume for further processing if elution volume was greater than 20 µL. Multiple tubes may be combined during concentration step (dependent upon case information).

5.6 Operation of the EZ1 advanced XL BioRobot

5.6.1 Cleaning/Maintenance – All cleaning/maintenance tasks shall be documented on the Forensic Biology Section EZ1&EZ2 Cleaning/Maintenance Form (located with the instrument). The QCO shall retain such information in the QC files with the specific instrument cleaning/maintenance records. The forensic scientist shall ensure that the Cleaning/Maintenance form is updated prior to use. If the instrument is not used for a week (e.g., due to being out of service) the Forensic Scientist assigned as instrument operator shall ensure that the reason is documented in the comments block on the Cleaning/Maintenance form.

5.6.1.1 UV Decontamination

5.6.1.1.1 Prior to use of the EZ1 Advanced XL for a batch, each Forensic Scientist shall perform a UV decontamination run as follows.

5.6.1.1.2 Switch on the EZ1 Advanced XL at the rear power switch.

5.6.1.1.3 Ensure the EZ1 Advanced XL door is closed. In the main menu, press "1" to select the UV light function.

5.6.1.1.4 Use the keys "0" through "9" to set the duration of the decontamination time to 30 minutes. Note: The default is 30 minutes. Pressing "ESC" will abort the procedure and return you to the main menu. After setting a valid time, press the "ENT" key.

5.6.1.1.5 Press "START" to switch on the UV lamp. The worktable will move slowly back and forth under the UV light. For user safety, the UV lamp cools for approximately 3 minutes. The EZ1 Advanced XL cannot be opened until after the cooling time has elapsed. After the cooling period, press the "ESC" key to return to the main menu.

5.6.1.2 Daily Cleaning (Post-Use) - After each protocol run, the piercing unit of the pipettor head and the worktable shall be cleaned by the Forensic Scientist as follows:

5.6.1.2.1 After removing the sample elution tubes, remove sample preparation waste (sample tubes, reagent cartridges, and filter tips), and discard in a biohazard waste container.

5.6.1.2.2 Close the EZ1 Advanced XL door.

5.6.1.2.3 Press "2" in the main menu to select the manual function.

5.6.1.2.4 Press "3" to choose the "Clean" operation.

5.6.1.2.5 Press "START". The EZ1 Advanced XL will lower the piercing unit.

5.6.1.2.6 Open the EZ1 Advanced XL door and wipe the piercing units and tray using isopropanol or other approved decontamination solution.

5.6.1.2.7 Close the EZ1 Advanced XL door and press "ENT". The piercing unit returns to its original position.

5.6.1.2.8 Press "ESC" to return to the main menu.

5.6.1.2.9 Open the EZ1 Advanced XL door. Clean the racks with isopropanol or other approved decontamination solution.

5.6.1.2.10 A new protocol can now be performed, or the instrument can be switched off.

5.6.1.3 Weekly Maintenance

5.6.1.3.1 During weeks where the instruments are in use, the Forensic Scientist shall apply silicon grease to the end of a filter-tip after ensuring the completion of the daily cleaning.

5.6.1.3.2 Apply silicon grease to the surface of the O-rings.

5.6.1.3.3 Place the tip onto the pipettor head and rotate the tip on the pipettor head to distribute the silicon grease evenly. Note: The filter-tip should sit flush against the upper white plastic bar if the O-rings are properly greased. There should not be a gap.

5.6.2 Operation of the EZ1 Advanced XL for DNA Purification

5.6.2.1 If fewer than 14 samples are being purified, the reagent cartridges, sample tubes, elution tubes, and filter-tips may be loaded in any order on the rack. However, tips and tubes must be in line with the reagent cartridges.

5.6.2.1.1 Reagent cartridges must be examined for precipitate in the wells. To dissolve any precipitate, heat the cartridges to ~37 ℃ for 5-10 minutes and agitate gently to re-suspend.

5.6.2.2 Switch on the EZ1 Advanced XL at the rear power switch. After initialization, the main menu appears.

5.6.2.3 Setup the worktable to perform the appropriate DNA Purification Protocol.

5.6.2.3.1 Remove the tip rack and cartridge rack from the worktable.

5.6.2.3.2 Load the reagent cartridges.

5.6.2.3.2.1 Invert the reagent cartridges several times to mix the magnetic particles. Use the same number of reagent cartridges as the number of samples being processed.

5.6.2.3.2.2 Tap the reagent cartridges until the reagents are deposited at the bottom of the wells.

5.6.2.3.2.3 Slide the reagent cartridges into the cartridge rack in the direction of the arrow until you feel resistance. Press down the cartridges until they click into place.

5.6.2.3.2.4 Return the cartridge rack to the worktable.

5.6.2.3.3 Load the elution tubes, filter-tips, and sample tubes.

5.6.2.3.3.1 Place the appropriate number of filter-tips into the tip holders.

5.6.2.3.3.2 Load the elution tubes, tip holders containing filter-tips and sample tubes into the sample rack in the following order:

Row 4: Sample Tube (containing digested sample)
Row 3: Empty
Row 2: Tip holder containing filter-tip
Row 1: Elution tube

Ensure that the elution tubes, tip holders containing filter-tips and sample tubes are loaded in the same order as the reagent cartridges.

Note: If the sample tube is a flip-top tube, the top shall be cut off prior to placing it onto the instrument.

5.6.2.3.3.3 Return the tip rack to the worktable.

5.6.2.3.3.4 Close the instrument door.

5.6.2.4 Starting and Finishing a Protocol Run.

5.6.2.4.1 Press "START" on the control panel. (If asked to "create a report file" choose "ESC".)

5.6.2.4.2 Press "1" (Trace Protocol) or "3" (Large Volume).

5.6.2.4.3 Press "2" to elute the samples in TE buffer. Then select the appropriate elution volume for the set of samples being run by pressing "2" (50), "3" (100) or "4" (200).

Note: Additional elution volumes may be used with written permission from the Technical Leader.

5.6.2.4.4 Press "ENT" to proceed through the text shown on the display, ensuring the worktable is setup as described.

5.6.2.4.5 Press "START" to begin the purification procedure.

5.6.2.4.6 When the protocol ends, the display shows "Protocol finished."

5.6.2.4.7 Press "ENT" to continue. Open the instrument door.

5.6.2.4.8 Check the elution tube to ensure proper volume of sample has been dispensed. If insufficient volume or other possible sample issue (e.g., smeared beads in pipette tip) is noted, the extract (and associated blank) may be returned to the elution tube and reprocessed with a new cartridge and pipette tip (if needed.) Document any additional steps for the sample in the case notes.

5.6.2.4.9 Retrieve the elution tubes containing the purified DNA and cap with screw caps. The DNA is ready to use or can be stored refrigerated at 2-8 °C until quantitation.

5.6.2.4.10 Press "ESC" twice to return to the main menu. Clean the instrument following steps in section **5.6.1.2.**

5.7 Operation of the EZ2 Connect Fx BioRobot

5.7.1 Cleaning/Maintenance - All cleaning/maintenance tasks shall be documented on the Forensic Biology Section EZ1&EZ2 Cleaning/Maintenance Form (located with the instrument). The QCO shall retain such information in the QC files with the specific instrument cleaning/maintenance records. The forensic scientist shall ensure that the Cleaning/Maintenance form is updated prior to use. If the instrument is not used for a week (e.g., due to being out of service) the Forensic Scientist assigned as instrument operator shall ensure that the reason is documented in the comments block on the Cleaning/Maintenance form.

5.7.1.1 UV Decontamination

5.7.1.1.1 Prior to use of the EZ2 Connect Fx for a batch, each Forensic Scientist shall perform a UV decontamination run as follows:

5.7.1.1.2 Ensure the EZ2 door is closed. Switch on the EZ2 Connect Fx at the front power switch. The startup screen appears, and the instrument is initialized.

5.7.1.1.3 After the initialization, the Login screen appears. Tap the "User ID" field and enter "Analyst" using the on-screen keyboard. Leave the "Password" field blank. Tap "Log In". The Home screen appears if the login credentials are correct.

5.7.1.1.4 On the touch screen, tap "Maintenance", then tap "UV run" to select the UV light function.

5.7.1.1.5 Use the "+" and "-" symbols to select one decontamination cycle. Note: One decontamination cycle takes approximately 34 minutes to complete.

5.7.1.1.6 To begin the procedure, tap "Start". The UV lamp will move slowly back and forth across the worktable. The EZ2 Connect Fx cannot be opened until after the UV decontamination cycle is complete.

5.7.1.2 Daily Cleaning (Post-Use) – After each protocol run, the piercing unit of the pipettor head and the worktable shall be cleaned by the Forensic Scientist as follows:

5.7.1.2.1 After removing the sample elution tubes, remove sample preparation waste (sample tubes, reagent cartridges, and filter tips), and discard in a biohazard waste container.

5.7.1.2.2 Close the EZ2 Connect Fx door.

5.7.1.2.3 On the "Protocol Run Completed" screen, tap "Move Down". The instrument lowers the piercing unit of the pipettor head. Alternately, the "Daily Maintenance" screen can be selected from the home touchscreen by tapping "Maintenance", then "Daily Maintenance."

5.7.1.2.4 Open the EZ2 Connect Fx door and wipe the piercing units and tray using isopropanol or other approved decontamination solution.

5.7.1.2.5 Close the door and confirm the maintenance has been completing by activating the checkbox.

5.7.1.2.6 On the touchscreen, tap "Finish". The piercing unit returns to its home position.

5.7.1.2.7 Open the EZ2 Connect Fx door. Clean the racks with isopropanol or other approved decontamination solution.

5.7.1.2.8 A new protocol can now be performed, or the instrument can be switched off.

5.7.1.3 Monthly Maintenance

5.7.1.3.1 During the months where the instruments are in use, the Forensic Scientist shall apply silicon grease to the end of a filter-tip after ensuring the completion of the daily cleaning.

5.7.1.3.2 On the touchscreen, tap "Maintenance". Tap "Weekly Maintenance."

5.7.1.3.3 Apply a small amount of silicon grease onto the surface of the O-rings, by just using a fingertip moistened with grease.

5.7.1.3.4 Place a filter-tip onto the pipettor head and rotate the filter-tip on the pipettor head to distribute the silicon grease evenly. Note: the filter-tip should sit flush against the upper white plastic bar if the O-rings are properly greased. There should not be a gap.

5.7.1.3.5 Make sure that the O-rings are only moistened with grease and that there are no clumps of grease visible.

Note: Excess grease may cause the filter-tips to fall of the pipettor heads during a protocol run.

5.7.1.3.6 Tap "Done".

5.7.2 Operation of the EZ2 Connect Fx for DNA Purification

5.7.2.1 If fewer than 24 samples are being purified, the reagent cartridges, sample tubes, elution tubes, and filter-tips may be loaded in any order on the rack. However, tips and tubes must be in line with the reagent cartridges.

5.7.2.1.1 Reagent cartridges must be examined for precipitate in the wells. To dissolve any precipitate, heat the cartridges to ~37 °C for 5-10 minutes and agitate gently to re-suspend.

5.7.2.2 Switch on the EZ2 Connect Fx at the front power switch. After initialization, the Login screen appears. Tap the "User ID" field and enter "Analyst" using the on-screen keyboard. Leave the "Password" field blank. Tap "Log In". The Home screen appears.

5.7.2.3 Setup the worktable to perform the appropriate DNA Purification Protocol.

5.7.2.3.1 Remove the tip rack and cartridge rack(s) from the worktable.

5.7.2.3.2 Load the reagent cartridges.

5.7.2.3.2.1 Invert the reagent cartridges several times to mix the magnetic particles. Use the same number of reagent cartridges as the number of samples to be processed.

5.7.2.3.2.2 Tap the reagent cartridges until the reagents are deposited at the bottom of the wells.

5.7.2.3.2.3 Slide the reagent cartridges into the cartridge rack in the direction of the arrow until you feel resistance. Press down the cartridges until they click into place.

5.7.2.3.2.4 Return the cartridge rack(s) to the worktable.

5.7.2.3.3 Load the elution tubes, filter-tips, and sample tubes.

5.7.2.3.4 Place the appropriate number of filter-tips into the tip holders.

5.7.2.3.5 Load the elution tubes, tip holders containing filter-tips and sample tubes into the sample rack in the following order:

Row A: Sample Tube (containing digested sample)
Row B: Empty
Row C: Tip holder containing filter-tip
Row D: Elution tube

Ensure that the elution tubes, tip holders containing filter-tips, and sample tubes are loaded in the same order as the reagent cartridges.

Note: If the sample tube is a flip-top tube, the top shall be cut off prior to placing it onto the instrument.

5.7.2.3.6 Return the tip rack(s) to the worktable.

5.7.2.3.7 Close the instrument door.

5.7.2.4 Starting and Finishing a Protocol Run.

5.7.2.4.1 Tap "DNA" in the "Applications" pane on the touchscreen or a favorite protocol in the "Favorites" pane.

5.7.2.4.2 Tap "DNA Investigator Kit" in the "Select Kit" pane. After a kit is selected, the "Next" button is enabled.

5.7.2.4.3 To proceed to the "Select Protocol", tap "Next."

5.7.2.4.4 Tap "DNA Investigator Trace", "DNA Investigator Large Volume", or "DNA Investigator Large Volume – RT" in the "Select Protocol" pane.

5.7.2.4.5 After a protocol is selected, the "Next" button is enabled. To proceed to the "Define Parameters" step, tap "Next."

5.7.2.4.6 To set values for protocol parameters, tap the box next to each parameter in the "Define Parameters" pane.

5.7.2.4.7 Select "TE" to elute the samples in TE buffer. Select "Tip Rack" for rack type. Select "20", "25", "50", "100" or "200" for elution volume.

Note: Additional volumes may be used for elution with written permission from the Technical Leader.

Note: 20 µl elution volume shall be used for shell casings only.

5.7.2.4.8 After selections are made, the "Next" button is enabled. To proceed to "Select Sample Positions" step, tap "Next."

5.7.2.4.9 To select the positions of your samples, tap the relevant rows on the worktable diagram or tap the corresponding row numbers underneath the diagram. The selected positions are highlighted. To select or deselect all positions, use the "Select All" toggle.

5.7.2.4.10 After you select at least one sample position, the "Next" button is enabled. To proceed to the "Enter Sample IDs" step, tap "Next."

5.7.2.4.11 To generate Sample IDs, press the "Generate Missing Sample IDs" button.

5.7.2.4.12 After Sample IDs are generated, the "Next" button is enabled. To proceed to the "Load Cartridge Rack" step, tap "Next."

5.7.2.4.13 Ensure reagent cartridges are loaded in the appropriate positions. To proceed to the "Load Tip Rack" step, tap "Next."

5.7.2.4.14 Ensure sample tubes, tip holders containing filter-tips, and elution tips are loaded in the appropriate positions. To proceed to the start of the protocol, tap "Next."

5.7.2.4.15 Review protocol selections in the "Run Setup Selection Overview" screen.

5.7.2.4.16 Press "Skip Load Check" to begin the purification procedure.

5.7.2.4.17 When the protocol ends, the display shows "Protocol Run Completed."

5.7.2.4.18 Open the instrument door.

5.7.2.4.19 Check the elution tube(s) to ensure proper volume of sample has been dispensed. If insufficient volume or other possible sample issue (e.g., smeared beads in pipette tip) is noted, the extract (and associated blank) may be returned to the elution tube and reprocessed with a new cartridge and pipette tip (if needed.) Document any additional steps for the sample in the case notes.

5.7.2.4.20 Retrieve the elution tubes containing the purified DNA and cap with screw caps. The DNA is ready to use or can be stored refrigerated at 2-8 °C until quantitation.

5.7.2.4.21 Clean the instrument following steps in section **5.7.1.2**.

5.7.2.5 Recovery of DNA after a stopped run

5.7.2.5.1 If a protocol run has been aborted before being completed (e.g., a protocol was aborted due to an error or stopped by a user), it is possible to recover the samples and complete the DNA extraction protocol.

5.7.2.5.2 Refer to the display message on the screen of the instrument. Note the position of the sample and the step to be performed in the manual. Refer to the "EZ2 Connect Fx Recovery Procedure Instruction Manual" document for specific steps on recovery of DNA samples.

5.8 Operation of the QIAsymphony SP

5.8.1 Cleaning/Maintenance – All cleaning/maintenance tasks shall be documented on the Forensic Biology Section QIAsymphony Cleaning/Maintenance Form (located with the instrument). The

QCO shall retain such information in the QC files with the specific instrument cleaning/maintenance records.

The forensic scientist shall ensure that the Cleaning/Maintenance form is updated prior to use. If the instrument is not used for a week (e.g., due to being out of service) the Forensic Scientist assigned as instrument operator shall ensure that the reason is documented in the comments block on the Cleaning/Maintenance form.

5.8.1.1 Daily Cleaning (Post-Use) – After each set of protocol runs, the worktable shall be cleaned by the Forensic Scientist as follows:

5.8.1.1.1 Remove sample tubes and discard in a biohazard waste container.

5.8.1.1.2 Remove exhausted reagent cartridges and discard in a biohazard waste container.

5.8.1.1.3 Remove full unit boxes from the "Waste" drawer, cap, and discard in a biohazard waste container. Move partial waste unit boxes to back of drawer. Place empty unit boxes in open slots.

5.8.1.1.4 Check liquid waste container, if liquid is above marked line discard liquid waste in appropriate hazard waste container marked for QIAsymphony waste.

5.8.1.1.5 Empty tip collection box.

5.8.1.1.6 Clean any active spills from the worktable using Decon-Quat, or similar.

5.8.2 Operation of the QIAsymphony SP for DNA Purification

5.8.2.1 Ensure that the QIAsymphony SP is switched on.

5.8.2.2 Load the required elution rack into the "Eluate" drawer.

5.8.2.2.1 Load the elution tubes or 96-well plate into the appropriate elution rack(s).

5.8.2.2.2 If using a 96-well plate, load the appropriate cooling adapter into Elution slot 1.

5.8.2.2.3 If using 1.5 mL screw cap tubes, load the appropriate adapters into Elution slots 1, 2, 3, or 4, depending on the number of samples.

Note: 1.5 mL screw cap tubes should not be loaded into elution slots 2, 3, or 4 if the instrument will be running overnight due to the possibility of sample evaporation.

5.8.2.3 Ensure the "Waste" drawer contains sufficient empty unit boxes for plastic waste generated during the protocol run. Perform an inventory scan of the "Waste" drawer, including the tip chute and liquid waste. Replace the tip disposal bag if necessary.

Note: Ensure the covers of the unit boxes are removed before loading the unit boxes into the "waste" drawer. If you are using 8-Rod Cover boxes for collecting used sample-prep cartridges and 8-Rod Covers, ensure that the box spacer has been removed.

5.8.2.4 Load the required reagent cartridge(s) into the "Reagents and Consumables" drawer.

5.8.2.4.1 Reagent cartridges must be examined for precipitate in the reagent troughs. To dissolve any precipitate, heat the cartridges to ~37 °C for 30 minutes and agitate gently to re-suspend.

5.8.2.4.2 Partially used reagent cartridges can be reclosed with Reuse Seal Strips for later reuse. Reagent cartridges can be open for a maximum of 15 hours and must be used within 14 days of opening.

5.8.2.4.3 When using a reagent cartridge for the first time:

5.8.2.4.3.1 Ensure the magnetic particles are fully resuspended by removing the magnetic-particle trough from the reagent cartridge frame. Vortex vigorously for at least 3 minutes. Replace the magnetic-particle trough in the reagent cartridge frame.

5.8.2.4.3.2 If not already done, dissolve the lyophilized carrier RNA in 1.6 ml Buffer ATE. Vortex briefly.

5.8.2.4.3.3 Transfer 400 µL of dissolved carrier RNA to each of the tubes in positions 1 and 2 of the enzyme rack on the reagent cartridge. Add an additional 1.2 mL Buffer ATE to each tube and mix by pipetting up and down several times.

5.8.2.4.3.4 Place the piercing lid on top of the reagent cartridge.

Note: The piercing lid is sharp. Take care when placing it onto the reagent cartridge. Make sure to place the piercing lid onto the reagent cartridge in the correct orientation.

5.8.2.4.3.5 Remove seal from magnetic-particle trough.

5.8.2.4.4 When using a partial, previously used reagent cartridge:

5.8.2.4.4.1 Remove the magnetic-particle trough from the reagent cartridge frame. Vortex briefly. Replace in the reagent cartridge frame.

5.8.2.4.4.2 Remove Reuse Seal Strip from each reagent trough.

5.8.2.4.5 Place the enzyme rack with the diluted carrier RNA into the reagent cartridge holder.

5.8.2.4.5.1 Remove lids from tubes 1 and 2 in the enzyme rack.

5.8.2.4.6 Place the reagent cartridge into the reagent cartridge holder.

5.8.2.4.7 Place reagent cartridge holder into either slot 1 or 2 in "Reagents and Consumables" drawer.

5.8.2.4.8 If using a High Efficiency (HE) assay control set, load TopElute Fluid (TOPE).

5.8.2.4.8.1 Navigate to the "R+C" menu on the QIAsymphony touchscreen.

5.8.2.4.8.2 With the "Reagents and Consumables" drawer open, select "Bottle ID".

5.8.2.4.8.3 Enter the barcode number from the TopElute Fluid bottle. The number may entered either manually or with the barcode scanner. Press "OK".

5.8.2.4.8.4 Remove cap from TopElute Fluid.

5.8.2.4.8.5 Place bottle of TopElute Fluid in "Reagents and Consumables" drawer.

Note: Ensure that the bottle of TopElute Fluid (TOPE) is scanned, opened, and placed into the "Reagents and Consumables" drawer before starting the inventory scan. Otherwise, the inventory scan must be repeated after scanning, opening, and placing in the drawer.

5.8.2.4.9 Load the required plastic ware into the "Reagents and Consumables" drawer.

5.8.2.4.9.1 Place the appropriate number of 200 and 1500 µL filter tips into the "Reagents and Consumables" drawer.

Note: Tip rack slots on the QIAsymphony worktable can be filled with either type of tip rack. The QIAsymphony SP will identify the type of tips loaded during the inventory scan.

5.8.2.4.9.2 Place the appropriate number of Sample Prep Cartridges, 8-well cartridges into the "Reagents and Consumables" drawer.

5.8.2.4.9.3 Place the appropriate number of 8-Rod Covers into the "Reagents and Consumables" drawer. Partial unit boxes should be placed at the back of the drawer.

Note: Do not combine partial Sample Prep Cartridges or 8-Rod covers

5.8.2.4.10 Perform an inventory scan of the "Reagents and Consumables" drawer.

5.8.2.5 Place samples into the appropriate sample carrier and load them into the "Sample" drawer.

5.8.2.6 Starting and Finishing a Protocol Run

5.8.2.6.1 On the "Sample Preparation" screen, select the batch to be queued.

5.8.2.6.2 On the "Sample Tube Selection Screen", ensure the proper tube type is selected, then press "Next".

5.8.2.6.3 Assign the assay control set to samples for the batch based on the type of sample being processed.

Sample Type - Starting Volume	Elution	Application	Assay Control Set
Question – 200 µl	1.5 mL tube	DNA Investigator	CW200_ADV_HE_V10
Question – 200 µl	96-well plate	Custom Investigator	CW200ADVHE_CR24547_ID5606

Question – 500 µl	1.5 mL tube	DNA Investigator	CW500_ADV_HE_V10
Question – 500 µl	96-well plate	Custom Investigator	CW500ADVHE_CR24547_ID5607
Known – 500 µl	1.5 mL tube or 96-well plate	DNA Investigator	Reference_500_V6

5.8.2.6.4 Select elution volume and output position in the elution drawer, then press "Queue".

5.8.2.6.5 When all batches have been queued, select "Run" from the "Overview" screen.

5.8.2.6.6 When all sample batches have been completed, open the eluate drawer.

5.8.2.6.7 On the Eluate Drawer/Elution Slot screen, select the elution slot from which the elution rack should be removed. Press the "Remove" button in the "Configure" tab to remove the elution rack from inventory. Press "Yes" to continue. To unload additional elution racks, repeat the process.

5.8.2.6.8 Retrieve the elution tubes/96-well elution plate containing the purified DNA. The DNA is ready to use or can be stored refrigerated at 2-8 °C until quantitation. If storing prior to quantitation, cap the elution tubes/96-well plate for storage.

5.8.2.6.9 Check the elution tubes/96-well elution plate to ensure proper volume of sample has been dispensed and sample has not evaporated if left on the instrument overnight.

5.8.2.6.10 If the reagent cartridge is only partially used, seal it with the Reuse Seal Strips and close the carrier RNA tubes with screw caps immediately after the end of the protocol run to avoid evaporation.

5.8.2.6.11 On the side of the partially used reagent cartridge, write the amount of time (in hours) the reagent cartridge was open, the number of samples remaining, analyst's initials, and the date.

5.8.2.6.12 Discard used sample tube, plates, and waste. Clean the instrument following the steps in section **5.8.1.1**.

5.8.2.6.13 Close the workstation drawers.

5.8.3 Recovery of DNA After a Stopped Run

5.8.3.1 If a sample batch has been aborted before being completed (e.g., a batch was aborted due to an error or a batch was stopped by a user), it is possible to recover the samples and complete the DNA extraction protocol.

5.8.3.2 Refer to the "QIAsymphony SP Recovery Procedure DNA Investigator ADV (Advanced) HE (High Efficiency) Application document for specific steps on recovery of DNA samples.

5.9 Concentration of Extracted DNA

NOTE: This procedure may be used if (1) the original final volume of the DNA extract, based on the training and experience of the Forensic Scientist, leaves the extract too diluted to obtain a DNA profile or (2) if the sample amount for an item required the use of multiple tubes and the final product shall be concentrated into one final tube. If the final volume is less than the final volume of the associated negative extraction control, the control shall also be concentrated using the steps below

5.9.1 Microcon Concentration

5.9.1.1 Vortex the extracted DNA and centrifuge briefly at maximum speed (5 seconds).

5.9.1.2 Wet the membrane of a new, labeled Microcon 100 concentrator (or equivalent) with TE. Transfer the extracted DNA to the concentrator.

5.9.1.3 Cap the concentrator and spin in a microcentrifuge at 4000 rpm for 10 minutes.

5.9.1.4 Remove the spin cap and add a measured volume of TE. The TE cannot be less than 20 µL and is dependent on the results of the previous quantitation. If the sample could be processed for Y-STR analysis, bring it up in no less than 50 µL of TE unless there is original evidence remaining. Remove the concentrator from the corresponding microcentrifuge tube and invert the concentrator onto a labeled microcentrifuge tube. Discard the corresponding centrifuge tube.

5.9.1.5 Spin the assembly in a mirocentrifuge at 4000 rpm for 5 minutes.

5.9.1.6 Discard the concentrator. Cap the microcentrifuge tube.

5.9.2 The speedvac concentrator may alternatively be used to concentrate samples (and associated controls) following the steps to dry extracts down in the Forensic Biology Evidence Handling procedure. The extract volume must be checked to ensure the proper final volume is reached.

5.10 Storage of DNA Extracts – Store the samples at 4 °C (short term) or frozen (long term). Prior to use of samples after storage, they shall be vortexed and then centrifuged for 5 seconds.

6.0 Limitations – N/A

7.0 Safety

7.1 Do not use bleach to clean or disinfect the instrument. Bleach in contact with salts from the buffers may produce toxic fumes.

7.2 Use caution when performing the daily cleaning on EZ1 Advanced XL and EZ2 Connect Fx instruments. Piercing units are sharp.

7.3 Use caution when placing the piercing lid on top of the QIAsymphony reagent cartridge, the piercing lid is sharp.

8.0 References

Forensic Biology Procedure for DNA Casework Training

9.0 Records

Forensic Biology Section Extraction worksheet (to be used in QC)
Forensic Biology Section EZ1&EZ2 Cleaning/Maintenance Form
Forensic Biology Section QIAsymphony Cleaning/Maintenance Form

10.0 Attachments

EZ2 Connect Fx Recovery Procedure Instruction Manual
QIAsymphony SP Recovery Procedure for DNA Investigator ADV (Advanced) HE (High Efficiency) Applications.

Revision History		
Effective Date	Version Number	Reason
02/07/2023	1.0	Initial document.