MNPD Crime Laboratory

Forensic Biology Technical Procedures Manual

Metropolitan Government of Nashville & Davidson County

Police Department

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1. Alternative Light Sources (ALS)

1.1 Scope

1.1.1 To describe the process by which items of evidence (i.e., clothing, bedding, etc.) are screened for the presence of potential human body fluids utilizing either the Leeds Spectral Vision (LSV) or UltraLite ALS® Turbo.

1.2 LSV Equipment/Materials/Reagents

1.2.1 LSV2 System hardware, including ALS/Imaging Arm and Mobile CPU Station

1.2.2 LSV System Software, Version 4.4

1.2.3 Black permanent marker for light colored evidence

1.2.4 Silver permanent marker for dark colored evidence

1.2.5 Various scales (fluorescent, dark colored, light colored) for reference sizing

1.2.6 Sterilizing surface cleaner, such as 10% Bleach Solution

1.2.7 Protective goggles, included with LSV

1.3 UltraLite ALS® Turbo Equipment/Materials/Reagents

1.3.1 UltraLite ALS® Turbo

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1.3.3 Head Assemblies (BMT, UV and IR)

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1.3.5 ALS amber glasses

1.3.6 Black permanent marker for light colored evidence

1.3.7 Silver permanent marker for dark colored evidence

1.3.8 Sterilizing surface cleaner, such as 10% Bleach Solution

1.4 Standards and Controls

1.4.1 Reference examples of known sample origin (blood and/or semen) will be viewed under the LSV or UltraLite ALS® Turbo to confirm fluorescence or absorbance as a positive control. Also viewed will be a reference substrate blank to serve as a negative control.

1.5 LSV Procedure

1.5.1 Preparation for LSV Screening

1.5.1.1 Clean the tabletop with a bleach solution. Optional: Butcher paper may then be used to line the tabletop.

1.5.1.2 For large/bulky evidence, clean the horizontal bar with a bleach solution, followed by an ethanol wipe.

1.5.1.3 Place evidence to be screened on tabletop or hang on horizontal bar.

1.5.1.4 Unlock the computer and turn on the green power button on the LSV base.

1.5.1.5 Double click the LSV Software icon.

1.5.1.6 Center the LSV head over the evidence. Adjust LED lights, located to either side of the LSV head, so that both light beams are coming together. These will need to be adjusted as the LSV head is moved up and down during screening.

1.5.1.7 Adjust room lighting with the dimmer switch located beside the door.

1.5.1.8 After examination is complete, close the LSV software and restart the computer. After restarted, lock the computer, and turn off the LSV base.

1.5.2 Positioning the LSV Head

1.5.2.1 The LSV head can be positioned in three ways: pitch, yaw, and roll.

1.5.2.2 To adjust the pitch, locate the silver Pitch Home Locking Pin on the left side of the LSV head. Pull out the pin and rotate it 90° to unlock the head for adjusting. Position the head up or down as desired, and lock it in to place using the LSV Head Positioning Lock handle, located on the right side of the head.

Figure 16: Pitch Home Lock Release button

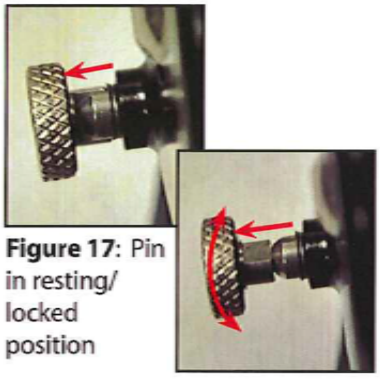


Figure 17: Pin in resting/ locked position

Figure 18: Pin pulled out and rotated 90°

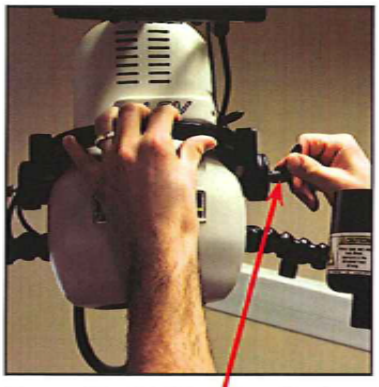


Figure 19: LSV Head Positioning Lock Handle

1.5.2.3

1.5.2.4 To adjust the yaw, locate the Yaw Lock on the right side of the LSV head. Loosen the lock to allow side to side movement. Position the head left or right as desired and tighten Yaw tension handle.

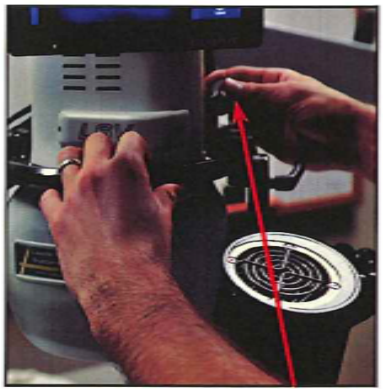


Figure 20: Yaw Tension Handle

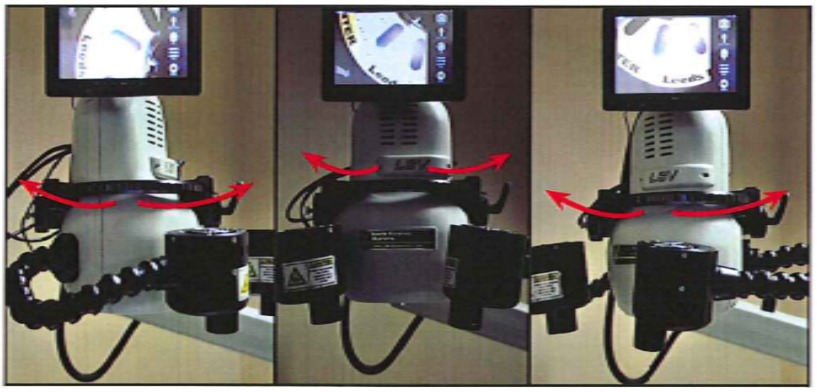


Figure 21: Adjust LSV head yaw left-to-right as desired.

1.5.2.5

1.5.2.6 To adjust the roll, locate the silver Roll Home Locking Pin located on the back of the black LSV head frame. Pull out the pin and rotate it 90° to release head for adjustment. Turn head clockwise or counter clockwise to desired position, and lock in place using the LSV Head Positioning Lock handle. This handle can be turned clockwise to tighten and counter clockwise to loosen.

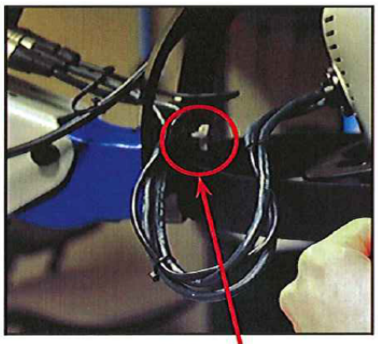


Figure 22: Roll Home Lock Release button

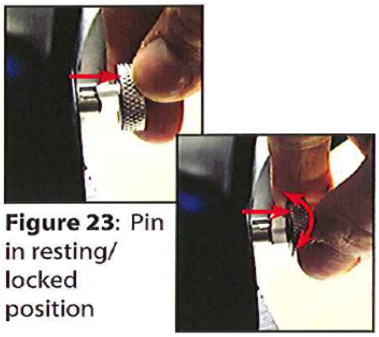


Figure 23: Pin in resting/ locked position

Figure 24: Pin pulled out and rotated 90°

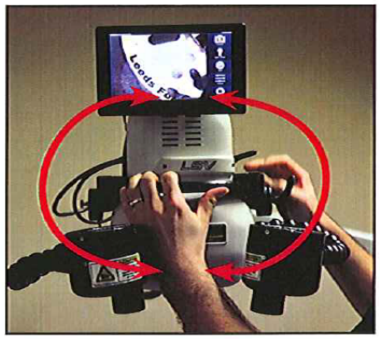


Figure 25: Adjust Roll of LSV Head to desired position

Figure 26: Adjust Roll of LSV Head to desired position

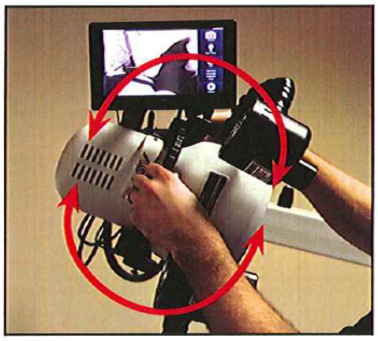


Figure 27: Adjust Roll of LSV Head to desired position

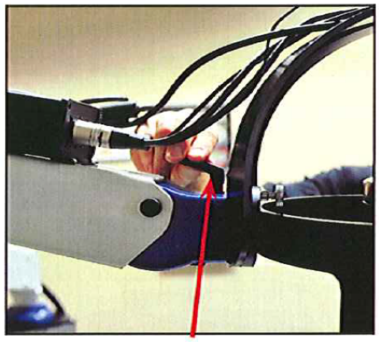


Figure 28: LSV Head Positioning Lock Handle

1.5.2.7

1.5.3 Light and Filter Controls

1.5.3.1 The LSV has seven LED light settings, ranging from 365 nm to 850 nm wavelengths, and a five position filter cassette, with filters ranging from 400 nm (clear) to 830 nm. This creates multiple light and filter options for viewing evidence, each of which will cause potential stains to look different. Each background material (ex. black t-shirt) and suspected stain type will need to be considered when choosing a light/filter combination.

1.5.3.2 Table 1 displays recommended light and filter settings, along with interpretation guidelines at the various settings.

1.5.3.2.1 **Table 1: Recommended Wavelength/Filter Settings**.

Body Fluid	Type of Fabric	Wavelength	Filter	Positive Result	Negative Result
Blood	White/Light colored	White/IR	Clear/ IR:830nm	Absorbance Observed	No Absorbance Observed
Blood	Dark colored, denim, and thick	IR	IR:830nm	Absorbance Observed	No Absorbance Observed
Semen, Saliva, Sweat, and Vaginal Secretions	All	Blue	Orange	Fluorescence Observed	No Fluorescence Observed

1.5.4 Camera Controls

1.5.4.1 There are two modes available in the Camera tab – Manual Exposure and Auto Exposure. In Manual Exposure mode, the LSV's shutter time is controlled by the user. In Auto Exposure mode, the LSV's software establishes the optimum exposure setting, allowing the user to control "Brightness" rather than "Shutter Time". In both modes, the "Gain" slider can be used to optimize frame rate. The White Balance button averages a whole image white balance each time it is clicked.

1.5.5 Lens Controls

1.5.5.1 Focus and Zoom are controlled in this tab, along with Aperture and Diopter Lens. Aperture controls how much light is allowed through the rear focal plane of the zoom optic. It also affects contrast, resolving power, and depth of focus. Diopter lenses allow for greater resolution at different working distances. The +3.5 diopter lens optimizes working distances between approximately 7 and 9 inches. The +2 diopter lens optimizes working distances between approximately 12 to 18 inches. The +1 diopter lens optimizes working distances of approximately 19 to 38 inches. With no diopters engaged, the LSV has a parfocal zoom with a working distance of approximately 40 inches and above.

1.5.5.2 **NOTE**: Light, filter, camera, and lens controls will need to be adjusted to maximize imaging for each piece of evidence. Presets, which allow all settings to be saved, can be made for classes of evidence. Refer to Table 2 for current MNPD presets. Save a preset by clicking "Save" under Preset dropdown menu. Enter the name for the preset, check the boxes beside the options desired, and click "OK".

1.5.5.2.1 **Table 2: MNPD Presets**.

LSV Preset Names	Light	Filter	Filter Color
Fluorescence	Blue-455	4-550nm	Orange
Fluorescence (2)	Violet-405	4-550nm	Orange
Alt. Fluroescence	Blue-455	2-610 nm	Red
Infared	IR-850	1-830nm	IR
Blood Absorbtion	Violet-400	2-610nm	Red
Blood Absorbtion 2	Violet-400	5-400nm	Clear
Visible	White-5600	5-400nm	Clear
White Balance Calibration	White-5600	5-400nm	Clear

1.5.6 LSV Screening

1.5.6.1 View the surface of the evidence, using a permanent marker to circle or otherwise indicate any stains or group of stains found. The LSV software allows annotation directly on the image to allow the recording of the analyst's initials, incident/Lab #, item/stain #, light and filter used, etc.. Below is a list of the options found on the Annotation and Measurement Toolbar.

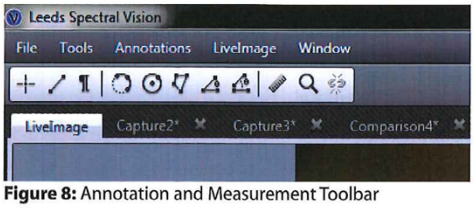


Figure 8: Annotation and Measurement Toolbar

Icon	Name	Function
	Crosshair	Insert a crosshair, defining a point of interest, into the image.
	Line	Insert a line between two points.
	Text	Insert a text window.
	Circle – Three Point	Insert a circle, based on three identified points on the circles circumference.
	Circle – Two Point	Insert a circle, based on the center of the circle and the radius.
	Polygon	Insert a polygon with a user defined number of points marking the outside perimeter.
	Angle – Three Point	Insert an angle based on a point on one arm, definition of the vertex, and a point on the second arm.
	Angle – Four Point	Insert an angle based on two points on the line of each arm.
	Calibration Line	Insert a line of known distance. This will calibrate all other measurements in the image as well. The line length must be defined and applied for calibrated measurements.
	Magnify	Digital zoom on the image.
	Link Images	Links two compared images so that when the field of view of one image is changed (i.e. Increased zoom or moved to display a different region of interest) that other image will change in the same way.

1.5.6.2 7

1.5.6.3 **Capturing, Saving and Exporting Images**.

1.5.6.3.1 In the upper right corner of the screen, click the "Capture" button, and click on newly created Capture tab. In the File dropdown menu, click "Save As". Then, click either "Export Raw Image" or "Export Annotated Image". In the pop-up box, navigate to the destination folder and appropriately name the saved image. A metadata text file with all image settings will automatically be generated and saved with the image file.

1.5.6.4 **Comparing Images**.

1.5.6.4.1 Live images and/or saved images can be viewed and saved in the same window for side-by-side comparison by clicking on "Compare To" under the Tools dropdown menu. Choose the image to be compared in the dropdown menu and click "OK". Click

on the newly created image tab with the two images side-by-side. This new image can be saved and exported as described above.

1.5.6.5 **Applying Settings**.

1.5.6.5.1 To use the same settings as a previously captured image, open the previously captured image and click the "Apply Settings" button on the Image Data panel located on the right-side panel. The live image will now have all the settings saved in the metadata file of the previously captured image.

1.6 UltraLite ALS® Turbo Procedure

1.6.1 Preparation for UltraLite ALS® Turbo Screening

1.6.1.1 Clean the tabletop with a bleach solution (Optional: Butcher paper may then be used to line the tabletop). Place the evidence to be screened on tabletop.

1.6.1.2 Take the UltraLite ALS® Turbo out of carrying case. Attach the desired Head Assembly to the UltraLite ALS® Turbo body. Insert the head in a straight and slow manner to avoid damaging the contact pins. Hold the head against the handle and rotate the locking ring on the handle until it is hand tight. DO NOT TWIST THE HEAD AND DO NOT OVERTIGHTEN. To remove the head, rotate the locking ring on the handle until the head becomes loose. Pull the head straight away from the handle.

1.6.1.3 Insert a battery pack into the bottom of the handle. The UltraLite ALS® Turbo features an energy conserving "sleep mode." After five minutes of inactivity, the unit will automatically shut off the indicator lights and enter this mode. If the unit is in sleep mode, press the on/off button once to "wake up" the unit. Press the on/off button once again to activate the light.

1.6.1.4 To increase the power output to the desired setting, press the Power Setting button on the top of the unit. The power increases from setting 1 to 2 to 3 to 4, then cycles back to 1. The green lights on the neck of the unit indicate the power setting that is currently in use. Light settings may need to be adjusted to maximize imaging for each piece of evidence.

1.6.1.5 Press the on/off button to deactivate the light.

1.6.1.6

1.6.1.7 Adjust the room lighting as necessary with the dimmer switch located beside the door.

1.6.1.8 NOTE: The yellow light on the neck will illuminate when the battery pack has 20% of its full charge remaining. Actual time remaining will vary, depending on which Head Assembly is in use.

1.6.1.9 Table 2: Recommended Wavelength/Filter Settings

LED Head Assembly	Body Fluid	Wavelength (nm)	Glasses	Positive Results	Negative Result
BMT	Semen, saliva, sweat, and vaginal secretions	450 nm	Amber	Fluorescence observed	No fluorescence observed
UV	Semen, saliva, sweat, and vaginal secretions	400 nm	Amber, Red, Yellow	Fluorescence observed	No fluorescence observed
IR	Blood	850 nm	Infrared detection cameras and other devices	Absorbance observed	No absorbance observed

1.6.1.10 **Charging the Battery Pack**

1.6.1.10.1 Plug the Battery Pack Charger into the wall and insert the cord jack into the side of the charger. Insert a battery pack into one of the battery docks. Make sure the battery is completely inserted and snaps in place.

1.6.1.10.2 The Battery Pack Charger features a logic circuit that will determine if a battery pack is in place and if it needs to be charged. If two battery packs are in place and both need charging, the unit will fully charge both batteries simultaneously. If all packs in the unit are fully charged, it will shut off until the charge falls below full. Each row of colored lights indicates the amount of charge each battery currently has, relative to a full charge.

1.6.2 UltraLite ALS® Turbo Screening

1.6.2.1 View the surface of the evidence, using a permanent marker to circle or otherwise indicate any stains or group of stains found.

1.6.2.2 After examination is complete, remove the battery pack from the UltraLite ALS® Turbo to conserve the life of the battery. To remove the battery pack, squeeze both tabs on the sides of the item, then pull straight out.

1.6.2.3 Store the UltraLite ALS® Turbo components in the travel case provided when not in use.

1.7 Limitations

1.7.1 The LSV and UltraLite ALS® Turbo are intended to identify areas for further testing, not definitive identification of stain type. This is because many other substances, such as bleach or brown paint, will mimic the appearance of body fluids under an ALS. Other screening techniques such as AP mapping or the testing of general swabbings may be used in conjunction with or in place of the LSV or UltraLite ALS® Turbo for difficult fabrics.

1.7.2 The MNPD-CL Forensic Biology Unit does not currently have the appropriate IR detection camera to utilize the IR Head Assembly for the UltraLite ALS® Turbo.

1.7.3 The UltraLite ALS® Turbo does not have image capturing capabilities to photograph the stains under the various light and filter settings.

1.8 Safety

1.8.1 Personal protective equipment must be used at all times. Protective goggles may be worn when using the ultraviolet (UV) light settings.

1.8.2 The LSV/Imaging unit is portable, but moving it requires a minimum of two people. Prior to moving the LSV/Imaging arm, collapse the arm, ensure that the magnetic lock is secure, and strap down the arm with the heavy-duty Velcro strap. Unplug and retract the power

cord into the LSV base. Use the orange wheels located on the caster feet to unlock the wheels. Remember to lock caster feet again when moving is complete.

1.8.3 The output intensity of the UltraLite ALS® Turbo solid-state emitters is strong enough to present the risk of injury to eye and skin tissue. Do not point the unit directly at the eyes or skin.

1.8.4 Use only accessories and power sources that are supplied with and designed specifically for the UltraLite ALS® Turbo. Do not allow moisture to collect on or enter the unit. Do not immerse. If the unit is used in humid environments, store in a warm, dry place between use to allow moisture to evaporate.

1.9 References

1.9.1 Performance Check of Leeds Spectral Vision (LSV) for Aiding Body Fluid Detection, Metro Nashville Police Department Crime Laboratory Forensic Biology Unit, June 2014

1.9.2 Performance Check of Leeds Spectral Vision System with adjusted LSV head and Leeds Software v4.0, Metro Nashville Police Department Crime Laboratory Forensic Biology Unit, June 2017

1.9.3 Leeds Spectral Vision System Instruction Manual (2016). Leeds Spectral Vision System. Leeds Forensics, Minneapolis, MN.

1.9.4 Leeds Vision Software Guide, Version 4.0 (2014). Leeds Spectral Vision System. Leeds Forensics, Minneapolis, MN.

1.9.5 Leeds Spectral Vision System Imaging Guide (2015). Leeds Spectral Vision System. Leeds Forensics, Minneapolis, MN.

1.9.6 UltraLite ALS® Turbo Operator's Manual (2011). UltraLite ALS® Turbo. CAO Group, Inc, West Jordan, UT.

1.9.7 Validation of UltraLite ALS® Turbo, Metro Nashville Police Department Crime Laboratory Forensic Biology Unit, January 2023. QMS WF 83525.

2. Phenolphthalein Test (Kastle-Meyer Test)

2.1 Scope

2.1.1 To describe the process by which evidence is screened for the potential presence of blood.

2.2 Equipment/Materials/Reagents

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

2.2.2 Microcentrifuge tubes (1.5mL or 2mL)

2.2.3 Kastle-Meyer Stock Solution (with zinc)

2.2.4 Ethanol

2.2.5 Amber dropper bottles

2.2.6 Sterile swabs

2.3 Standards and Controls

2.3.1 On each day the test will be utilized, a positive control (known blood sample) and a negative control (reagents only) must be tested and the expected results must be obtained.

2.3.2 The results of these controls must be documented in the casefile.

2.4 Sample Selection

2.4.1 Refer to the section Evidence Handling/Blood of the MNPD-CL Forensic Biology Quality Manual.

2.5 Procedure

2.5.1 A small cutting of stain or swabbing of a suspected bloodstain is placed into a microcentrifuge tube.

2.5.2 Two to three drops of ethanol are placed onto the stain.

2.5.3 Two to three drops of phenolphthalein (Kastle-Meyer Reagent Stock Solution) are added to the stain.

2.5.4 Wait a few seconds to ensure that no color develops at this stage, and then add two to three drops of 3% Hydrogen Peroxide.

2.5.5 Interpretation:

2.5.5.1 Positive: A pink color change up to 30 seconds after addition of hydrogen peroxide.

2.5.5.2 Negative: No observed color change or a color change after 30 seconds.

2.5.5.3 Inconclusive: A pink color change prior to the addition of hydrogen peroxide.

2.6 Limitations

2.6.1 The Kastle-Meyer test will give false positive results in the presence of some plant peroxidases, such as horseradish. The Kastle-Meyer test is also not specific to human blood and will react with meat and food products containing blood. As such, this test will not be considered a confirmatory test for the presence of human blood.

2.6.2 Chemical oxidants such as copper and nickel salts may cause the Kastle-Meyer reagent to turn pink prior to the addition of hydrogen peroxide; therefore, it is important to wait a few seconds prior to adding hydrogen peroxide during testing.

2.6.3 Certain factors may also prevent blood from producing a positive reaction. Limitations based on the sensitivity of the test may produce negative results when the blood is present at low concentrations. Depending on the substrate on which the stain is deposited, negative results were produced when blood was diluted greater than 1:6000 on filter paper and greater than 1:25000 on a swab according to an internal laboratory study. Washing, rain, heat, and time can reduce the concentration of blood.

2.6.4 Sensitivity limitations are not the only factors that can prevent a positive reaction. Studies have also shown that reducing agents can also interfere with the reaction involved in the Kastle-Meyer test. If reducing agents are present on the item in which the blood is deposited (i.e., metals) or the bloodstain comes into contact with a product with high reduction strength (i.e., certain detergents or foods), interference may occur.

2.7 Safety

2.7.1 Phenolphthalin stock solution is a skin and eye irritant. Hydrogen Peroxide is corrosive. Caution should be used when handling these chemicals and personal protective equipment should be used at all times.

2.7.2 Zinc's reaction with water or moisture in the air produces hydrogen which can react explosively with oxygen. Therefore, zinc should not be discarded in the wastebasket.

2.8 References

2.8.1 Performance Check of Phenolphthalein in the Presumptive Test of Blood, Metro Nashville Police Department Crime Laboratory Forensic Biology Unit, June 2014

2.8.2 Tobe, et al. Evaluation of Six Presumptive Tests for Blood, Their Specificity, Sensitivity, and Effect on High Molecular-Weight DNA, J Forensic Sci, January 2007, Vol. 52, No. 1, pg 102-109

2.8.3 Glaister, John. "The Kastle-Meyer Test for the Detection of Blood. Considered from the Medico-Legal Aspect." National Center for Biotechnical Information. www.ncbi.nlm.nih.gov. British Medical Journal, 10 Apr 1926.

2.8.4 Gaensslen, R.E. Sourcebook in Forensic Serology, Immunology, and Biochemistry, Unit II: Identification of Blood: 1989 Update (NCJ 160880), pp. 103-105.

2.8.5 Castello Ponce, Ana et al. Critical Revision of Presumptive Tests for Bloodstains, FBI Forensic Science Communications, July 1999, Volume 1, No. 2, https://archives.fbi.gov/archives/about-us/lab/forensic-science-communications/fsc/july1999/ponce.htm

3. HemaTrace Blood Test

3.1 Scope

3.1.1 To describe the process by which an evidentiary stain is identified as blood with the HemaTrace test.

3.2 Equipment/Materials/Reagents

3.2.1 Test device (individually packaged)

3.2.2 HemaTrace Extraction Buffer vial

3.2.3 Pipette and pipette tips

3.2.4 Scalpel/scissors

3.2.5 Timer

3.2.6 Transfer pipettes

3.3 Standards and Controls

3.3.1 In addition to lot number quality control testing, on each day that the test is utilized, a positive control (known human blood) and a negative control (extraction buffer only) must be tested, and the expected results must be obtained. The result of the daily testing must be recorded in the casefile. The control line in the control "C" area can be considered an internal procedural control.

3.4 Sample Selection

3.4.1 Refer to The Evidence Handling/Blood/Evidentiary Bloodstains Section of the MNPD-CL Forensic Biology Quality Manual.

3.5 Procedure

3.5.1 A small cutting or swabbing of a suspected blood stain is placed directly into a labeled HemaTrace extraction buffer vial.

3.5.2 Allow the sample to incubate at room temperature for 30 minutes. Sample may be left to incubate overnight (no longer than 24 hours).

3.5.3 Ensure the vial cap is tight and gently mix the contents for at least 10 seconds without causing the buffer to foam.

3.5.4 Verify the lot number of HemaTrace cards that will be used. Depending on which method is instructed by the manufacturer, add the following to the "S" sample well on the test device using the supplied dropper or a pipette:

3.5.4.1 ~2 drops, or ~80uL

3.5.4.2 ~6-7 drops, or ~200uL

3.5.5 Results can be read at 10 minutes. The minimum time in a positive result is the time at which both lines appear. For negative results, wait the full 10 minutes.

3.6 Interpretation

3.6.1 Positive: A positive result is indicated by two pink lines, one each in the "T" (test) area and in the "C" (control) area.

3.6.2 Negative: A negative result is indicated by a single pink line in the "C" (control) area. No line will be observed in the "T" (test) area.

3.6.3 Inconclusive: Regardless of the presence or absence of a line in the "T" (test) area, valid results will only be recorded if a pink line is present in the "C" (control) area. If no pink line is visible in the "C" area, the test should be repeated.

3.7 Limitations

3.7.1 Low concentration of blood may produce negative results. During an internal laboratory study, negative results were produced when blood was diluted greater than 1:1000. Washing, rain, heat, and time can reduce the concentration of blood.

3.7.2 The results of this test should not be read after 10 minutes since non-specific reactions may occur and can result in false positives.

3.7.3 Positive results may be obtained from whole blood from the domestic ferret and higher primates (Anthropoidea). Since the possibility of encountering a higher primate or ferret blood in routine casework is minimal and can be considered on a case-by-case basis, the fact that the kit cross reacts with these animals is not of great concern. If any doubt exists, the results can be confirmed by DNA analysis.

3.7.4 False negative results can be produced by "High Dose Hook Effect" when large amounts of hemoglobin are present in a sample. An approximate guide for to the hood effect is the color of the extract solution. The darker red, the greater the chance of a hook effect occurring. Ideally, for fresh bloodstains, the extract solution should be straw-colored. In

cases where the effect is strongly suspected, the extract may be retested using a 1:10 and/or a 1:100-fold dilution.

3.7.4.1 This test is not specific to human blood and is only considered a confirmatory blood test.

3.8 Safety

3.8.1 Caution should be used when handling kit reagents. Personal protective equipment should be used at all times.

3.9 References

3.9.1 ABAcard HemaTrace Technical Information Sheet, Catalog # 708424. Abacus Diagnostics, rev March 2020.

3.9.2 ABAcard HemaTrace Technical Information Sheet, Catalog # 708424. Abacus Diagnostics, rev December 2022.

3.9.3 ABAcard Hematrace Sensitivity Study, Metro Nashville Police Department Crime Laboratory Forensic Biology Unit, January 2023.

3.9.4 RSID Blood and ABAcard Hematrace Test Comparison Study, Metro Nashville Police Department Crime Laboratory Forensic Biology Unit, July 2014.

3.9.5 Validation Study of the Abacus Diagnostics ABAcard HemaTrace Membrane Test for the Forensic Identification of Human Blood. S. Johnston, J. Newman, R. Frappier. Canadian Society of Forensic Science, Vol 36, No 3 (2003), pg 173-183.

4. Acid Phosphatase Mapping Test

4.1 Scope

4.1.1 To describe the process by which evidence is preliminarily screened for the potential presence of semen stains.

4.2 Equipment/Materials/Reagents

4.2.1 AP Spray Working Solution (in spray bottle)

4.2.2 Filter paper

4.2.3 Marker/Sharpie

4.2.4 Autoclaved Ultrapure water

4.2.5 Timer

4.3 Standards and Controls

4.3.1 On each day the test will be utilized, a positive control (known semen sample) and a negative control (filter paper) must be tested, and the expected results must be obtained and documented in the casefile.

4.3.2 If the expected results are not obtained, the spray should be discarded and prepared again. The new spray will be tested against the positive and negative controls before being utilized in casework.

4.3.3 Note: Acid phosphatase is known to degrade after being deposited. Moisture and heat are the most deleterious factors, so known semen samples will be dried and stored at room temperature in a dark environment.

4.4 Sample Selection

4.4.1 Refer to The Evidence Handling/Suspected Semen Section of the MNPD-CL Forensic Biology Quality Manual.

4.5 Procedure

4.5.1 Obtain a piece of filter paper that is sized appropriately for the item being tested.

4.5.2 Lay the filter paper on the item or wrap the filter paper around the swab tip. Lines may be drawn on the paper and garment so that the orientation of the paper to the garment can be ascertained once the test is complete.

4.5.3 Wet the filter paper lightly with autoclaved ultrapure water.

4.5.4 Press the paper into the item for 1-2 minutes.

4.5.5 Remove the filter paper from the evidence. Place the filter paper in a fume hood and spray with the AP Spray Working Solution. Make sure to coat the paper evenly with the spray.

4.5.6 The result should be observed for up to 3 minutes for a pink/purple color to appear if acid phosphatase is present at significant levels.

4.5.7 Interpretation:

4.5.7.1 Positive: A pink/purple color appears within 3 minutes.

4.5.7.2 Negative: No color change appears within 3 minutes.

4.6 Limitations

4.6.1 Other bodily fluids, such as vaginal secretions and male urine, as well as bacteria, fungi, and some feminine hygiene products are known to produce a positive reaction utilizing this test. As such, this test will only be considered a presumptive test for the presence of semen.

4.6.2 Acid phosphatase is known to degrade after being deposited. Therefore, low level samples or samples with a long time frame between deposit and collection may be degraded beyond the limit of detection.

4.7 Safety

4.7.1 AP Spray Working Solution contains α-Naphthyl phosphate and Fast Blue B, which emit toxic fumes when exposed to heat. Caution should be used when handling these chemicals. All testing should be conducted in a fume hood and personal protective equipment should be used at all times.

4.8 References

4.8.1 Performance Check of Acid Phosphatase Tests for the Presence of Semen, Metro Nashville Police Department – Crime Laboratory Forensic Biology Unit, September 2014.

4.8.2 A Greenfield, M.A. Sloan, Identification of Biological Fluids and Stains, in: S.H. James, J.J. Nordby (Eds.), Forensic Science: An Introduction to Scientific and Investigative Techniques, CRC Press, Boca Raton, 2003, pp. 203-220.

4.8.3 E.L. Jones Jr., The Identification of Semen and Other Body Fluids, in R. Saferstein (Ed.), Forensic Science Handbook, Vol. II, Prentice Hall, Upper Saddle River, NJ, 2005, pp. 329-382.

4.8.4 Lewis, J., S. Jones, F. Baxter, A. Siemieniuk, and R. Talbot. "The Fallacy of the Two-minute Acid Phosphatase Cut off." Science & Justice 52.2 (2012): 76-80.

4.8.5 Redhead, Paul, and Melanie K. Brown. "The Acid Phosphatase Test Two Minute Cut-off: An Insufficient Time to Detect Some Semen Stains." Science & Justice 53.2 (2013): 187-191.

5. Microscopic Examination for Sperm

5.1 KPIC Staining

5.1.1 Scope

5.1.1.1 To describe the process by which a chemical stain is utilized to make the observation of potential spermatozoa more distinct.

5.1.2 Equipment/Materials/Reagents

5.1.2.1 Autoclaved Ultrapure Water

5.1.2.2 Microscope Slide

5.1.2.3 Glass Coverslips

5.1.2.4 Cytoseal or other mounting medium

5.1.2.5 100% Ethanol

5.1.2.6 Forceps

5.1.2.7 Scalpel/Scissors

5.1.2.8 Hot Plate

5.1.2.9 Picroindigocarmine Stain

5.1.2.10 Nuclear Fast Red Stain

5.1.3 Standards and Controls

5.1.3.1 New lots of Nuclear Fast Red and Picroindigocarmine stains will be tested against a known semen sample prior to use in casework. The reagents will be deemed acceptable for use if Nuclear Fast Red stains nuclear material red and Picroindigocarmine stains non-nuclear material green.

5.1.4 Sample Selection

5.1.4.1 Refer to The Evidence Handling/Suspected Semen Section of the MNPD-CL Forensic Biology Quality Manual.

5.1.5 Procedure

5.1.5.1 If the item being examined is an absorbent material, such as fabric, excise a small piece. For non-absorbing materials, collect the sample onto a sterile Copan swab. Copan swabs received in sexual assault kits will be applied directly to the microscope slides.

5.1.5.2 Wipe the microscope slide well with 100% ethanol and allow to fully dry before applying the sample to the slide.

5.1.5.3 Add 1-2 drops of autoclaved ultrapure water to a sampled area on the labeled (case # and sample ID #, initials, and date) microscope slide. Smear the cutting/swab in the water gently but firmly for 20 seconds, using forceps or scissors to grip the cutting. Cutting/swab may be used for p30 or DNA according to workflow in the MNPD-CL Forensic Biology Quality Manual/Evidence Handling/Suspected Semen Section.

5.1.5.4 Place the slide on a hot plate set to "2" for 20 minutes to allow the cellular material to heat fix to the slide.

5.1.5.5 Add enough Nuclear Fast Red stain to cover the cellular material on the slide (approx. 2 drops).

5.1.5.6 After 15 minutes, gently rinse the slide with autoclaved ultrapure water in a squeeze bottle.

5.1.5.7 Add enough Picroindigocarmine stain to cover the cellular material on the slide (approx. 2 drops).

5.1.5.8 After 15 seconds, gently rinse the slide with 100% ethanol in a squeeze bottle.

5.1.5.9 Once the ethanol has fully evaporated, add mounting medium to the stained smear and cover with a glass coverslip, being careful to minimize bubbles. Allow mounting medium to dry before placing slide on microscope.

5.1.6 Limitations

5.1.6.1 Nuclear Fast Red and picroindigocarmine stain other cellular material along with spermatozoa. The slide must be carefully looked at to distinguish sperm cells from white blood cells, yeast, and epithelial cells. In the event that a concordant conclusion cannot be drawn from both the analyst and the second reader or both individuals render the result inconclusive due to degradation, staining, and/or an obscurity that overlaps a single possible sperm, the microscopic examination will be reported inconclusive.

5.1.6.2 In addition, the chemicals used to stain will stain sperm from other species. However, the morphology of the sperm cells from other species is different and can be distinguished from human spermatozoa. Therefore, this protocol will be considered a confirmatory test for spermatozoa.

5.1.7 Safety

5.1.7.1 Picroindigocarmine contains picric acid, which is explosive when dry. Do not allow solution to dry out. Both stains are irritants and can be toxic. Caution should be used when handling these chemicals. Personal protective equipment should be worn at all times.

5.1.8 References

5.1.8.1 Performance Check of KPIC Staining for the Visualization of Sperm, MNPD-CL Forensic Biology Unit, September 2014.

5.1.8.2 Allery, J.P. et al. Cytological Detection of Spermatozoa: Comparison of Three Staining Methods. J Forensic Sci 2001; 46(2) pp.349-351.

5.1.8.3 Gaennslen, R.E. Sourcebook in Forensic Serology, Immunology, and Biochemistry. U.S. Dept. of Justice, National Institute of Justice, 1983. 149-155. Print.

5.2 Kohler Illumination

5.2.1 Scope

5.2.1.1 To describe the process by which even light distribution is achieved on the light microscope.

5.2.2 Equipment/Materials/Reagents

5.2.2.1 Compound Microscope

5.2.2.2 Microscope Slide

5.2.3 Procedure

5.2.3.1 Place a microscope slide onto the microscope stage and secure it using the specimen holders.

5.2.3.2 Turn the microscope on and adjust the light source to half of the potential brightness.

5.2.3.3 Using the 10X objective lens, focus the specimen using the coarse adjustment knob.

5.2.3.4 To adjust the eyepiece, close the right eye. Use the fine adjustment knob to sharpen the image. Then, close the left eye. Turn the Diopter ring located on the right eyepiece clockwise or counterclockwise to bring the specimen into focus.

5.2.3.5 Close the field diaphragm ring and the condenser aperture diaphragm ring. A small circle of light should be visible, which needs to be centered in the field of view. Adjust the condenser screws slowly to center the circle of light. If the circle of light is not visible,

open the field diaphragm ring until the circle of light is just visible. Then, adjust the condenser screws until the light is centered and then close the field diaphragm ring.

5.2.3.6 Adjust the condenser focus knob until the circle of light is sharp.

5.2.3.7 Remove the left eyepiece. Look down the eyepiece cylinder. Open the condenser aperture diaphragm ring slowly until the circle of light fills ¾ of the field. Replace the eyepiece and record the setting for the diaphragm.

5.2.4 References

5.2.4.1 Silverberg, A. "Easy Kohler Illumination Method." Trent University. Retrieved from www.trentu.ca, Nov. 2013.

5.2.4.2 Leica DM2000, DM2000 LED, DM2500, DM3000, DM3000 LED Operating Manual (2005). DM2000 LED Light Microscope. Leica Microsystems, Wetzlar, Germany.

5.3 Microscopic Examination for Spermatozoa

5.3.1 Scope

5.3.1.1 To describe the process by which chemically stained microscope slides are examined for the potential presence of spermatozoa.

5.3.2 Equipment/Materials/Reagents

5.3.2.1 Compound Microscope

5.3.2.2 Microscope Slide

5.3.3 Procedure

5.3.3.1 Place the slide on the stage of the microscope and examine the stained area using 400X magnification.

5.3.3.2 Start at one end of the stained area and proceed in a grid search fashion until the entire slide is examined. If the presence of spermatozoa is readily apparent, the entire slide does not need to be examined. In the case of a sample with 20 sperm heads or less, the sperm head count shall be noted in the case notes.

5.3.3.2.1 Note: If prone to motion sickness, it is recommended to examine the slide up and down instead of side to side.

5.3.3.3 At a minimum, it is required to capture the image of at least one sperm head to confirm the presence of semen. Refer to Section 5.4.3.2 for instructions on capturing an image.

5.3.4 Limitations

5.3.4.1 Refer to 5.1.6 of the MNPD-CL Technical Procedures Manual.

5.3.5 References

5.3.5.1 Performance Check of KPIC Staining for the Visualization of Sperm, MNPD-CL Forensic Biology Unit, September 2014.

5.3.5.2 Allery, J.P. et al. Cytological Detection of Spermatozoa: Comparison of Three Staining Methods. J Forensic Sci 2001; 46(2) pp.349-351.

5.3.5.3 Gaennslen, R.E. Sourcebook in Forensic Serology, Immunology, and Biochemistry. U.S. Dept. of Justice, National Institute of Justice, 1983. 149-155. Print.

5.4 Leica Application Suite Software

5.4.1 Scope

5.4.1.1 To describe the functionality of the Leica Application Suite Software v4.4 and describe the process of using the software when viewing evidence under the microscope.

5.4.2 Equipment/Materials/Reagents

5.4.2.1 Leica Application Suite Software v4.4

5.4.2.2 Microscope

5.4.3 Procedure

5.4.3.1 Functions of Software

5.4.3.1.1 For all buttons on the software, once clicked the button will turn red to indicate the function is active. If the button is black, the function is off.

5.4.3.1.2 CAMERA TOOLBOX: (Left to right)

5.4.3.1.2.1 **Automatic Exposure:** Proper exposure is maintained but the user can adjust the brightness and any hardware changes that could affect the exposure are compensated for automatically.

5.4.3.1.2.2 **Exposure Adjust Tab**:

5.4.3.1.2.2.1 **Brightness:** How light or how dark each color in the image is. Use small increases in brightness to help differentiate between colors; too much and detail begins to disappear.

5.4.3.1.2.2.2 **Saturation**: Determines the amount of each color that is present. Use this function to achieve color subtlety in the image.

5.4.3.1.2.2.3 **Gamma**: A value applied to color levels to compensate for different ways in which the image is viewed. Use this function to achieve a contrast 'match' to the specimen.

5.4.3.1.2.2.4 **Gain**: Only active when Automatic Exposure is disabled. A function for changing the brilliance of an image without changing the exposure. Start with a Gain value of 1.0 and gradually increase the value. Too high a Gain setting will 'bleach' the image, causing a loss of fine detail and may introduce 'noise'.

5.4.3.1.2.3 **Automatic White Balance:** All of the neutral tones – white through grey to black – are adjusted to remove any 'color' content to maintain a clean, well-defined image.

5.4.3.1.2.3.1 **NOTE**: If the image is too dark or too light Automatic White Balance may fail and an error message displayed. It may be possible to lighten or darken the image with Exposure Adjust controls or change the lighting conditions at the microscope.

5.4.3.1.2.4 **Easy Camera Control**: If the specimen is evenly and well lit, focus and contrast are acceptable, then the Easy Camera Control tool allows users to 'fine tune' the image and achieve even better results.

5.4.3.1.2.4.1 **Twain Interface**: Can be accessed by right-clicking on the easy button to display a drop-down menu. The Twain Interface shows the camera data and basic exposure settings on a single, compact display.

5.4.3.1.2.5 **Camera and Microscope Linking**: This function is Not Applicable to our microscopes and camera setups.

5.4.3.1.2.6 **Leica High Dynamic Range (HDR)**: Automatically captures a number of images each at different exposure, and then combining them digitally into a single image that balances the contrast range.

5.4.3.1.2.6.1 **Averaging**: Can be accessed by right-clicking on the HDR button to display a drop-down menu. This setting averages multiple exposures to reduce noise without reducing detail.

5.4.3.1.2.7 **Show Under/Over Exposure**: Gives a fast indication of those areas of the image that are not exposed properly.

5.4.3.1.2.8 **Camera Configuration**: Saves Input Settings that users load to use them on another occasion by selecting from a drop-down menu.

5.4.3.1.2.8.1 **NOTE**: When the viewer opens, the configuration defaults to the Last Used settings.

5.4.3.1.2.9 **Shading Configurations**: This function is Not Applicable to our microscopes and camera setups.

5.4.3.1.2.10 **Predefined Camera Setups**: Settings for the most common microscope contrast methods that you can quickly select and use.

5.4.3.1.2.11 **Reset Camera**: Provides a quick way of restoring the camera to factory default settings.

5.4.3.1.3 SIDE TOOL BAR: (Top to bottom)

5.4.3.1.3.1 **Scale Bar and Annotations**: Shows options for annotating an image. Basic annotations include image name, description, date and time, drawing a line, and editing the color and background of the text.

5.4.3.1.3.2 **Floating Navigator**: Click to enable Navigator to file folders.

5.4.3.1.3.3 **Panning**: Examine areas of images that extend beyond the viewer edges into the display area.

5.4.3.1.3.4 **Zoom In and Out**: In the displayed area

5.4.3.1.3.5 **Fit image to the Viewer area**: Causes the image to expand to the full size of the viewer.

5.4.3.1.3.6 **Display at Original Size**: Displays the image at its captured size.

5.4.3.1.3.7 **Form Data**: Shows the images associated metadata in a dock-able window.

5.4.3.1.3.8 **Show/Hide Image**: Displays or removes image from view.

5.4.3.1.3.9 **Show/Hide Data Grid**: Displays or removes image data grid.

5.4.3.1.3.10 **Show/Hide Thumbnail Gallery**: Displays or removes a gallery of captured images.

5.4.3.1.3.11 **Record Details**: Shows all the associated details about captured image.

5.4.3.1.3.12 **Select Visible Fields**: Allows user to select which details will be displayed when Record Details button is selected.

5.4.3.1.3.13 **Viewer Options**: Not Applicable with our microscope/camera setups

5.4.3.1.3.14 **Save Image**: Allows you to save the image.

5.4.3.2 Microscopes

5.4.3.2.1 Ensure the microscope is turned on. Pull the plunger, located under the eyepieces, out to allow viewing on the computer monitor.

5.4.3.2.2 Open the LAS v4.4 software.

5.4.3.2.3 Begin on the Acquire Workflow Tab and using the 400X magnification, bring the specimen into focus on the screen.

5.4.3.2.4 Using the software functions (refer to 5.4.3.1), white balance the image or select Auto Exposure. Fine tune the image as necessary.

5.4.3.2.5 Once it is determined that an image needs to be captured, ensure microscope is set to 40X objective lens and specimen is in focus on the screen.

5.4.3.2.6 Click Acquire Image to take a photo. Hit the Export button on the right-side panel. In the resulting dialog box, choose the folder to save the image, rename the image with its item number, and change the file type to save as a jpg.

5.4.4 References

5.4.4.1 Leica Application Suite LAS User Manual (2013). DM2000 LED Light Microscope. Leica Microsystems, Wetzlar, Germany.

5.4.4.2 Leica LED 1000 Operating Instruction (2009). DM2000 LED Light Microscope. Leica Microsystems, Wetzlar, Germany.

6. ABAcard® p30 Test

6.1 Scope

6.1.1 To describe a process by which evidence is screened for the potential presence of seminal fluid.

6.2 Equipment/Materials/Reagents

6.2.1 Test device (individually packaged)

6.2.2 p30 Buffer

6.2.3 Thermoshaker

6.2.4 Transfer pipette (included in kit)

6.2.5 Timer

6.2.6 Scalpel/scissors

6.2.7 Sample tube (1.5mL or 2.0mL)

6.3 Standards and Controls

6.3.1 In addition to lot number quality control testing, each lot of ABAcards® p30 must be tested using a positive control (known semen standard) and a negative control (p30 buffer) prior to use in casework each day. The result of the daily testing must be recorded in the casefile.

6.3.2 The control line in the control "C" area can be considered an internal procedural control.

6.4 Procedure

6.4.1 Place sample for testing into a sample tube.

6.4.2 If a full swab is used, add 500µl of p30 buffer and allow to incubate at room temperature for 30 to 120 minutes on a thermoshaker at room temperature (23°C) . If using a partial swab, add 250µl of p30 buffer and allow to incubate at room temperature for 30 to 120 minutes on a thermoshaker at room temperature (23°C).

6.4.3 For the 200ul test device: Pipette 200ul (~8 drops with transfer pipette) of supernatant into the sample well "S" on the test device.

6.4.4 For the 80ul test device: Pipette 80ul (~2 drops with transfer pipette) of supernatant into the sample well "S" on the test device.

6.4.5 Read results at 10 minutes.

6.4.6 Interpretation:

6.4.6.1 Positive: A positive result is indicated by a pink line in the "T" (test) and "C" (control) regions.

6.4.6.2 Negative: A negative result is indicated by a single pink line in the "C" (control) region. No line will be observed in the "T" (test) region.

6.4.6.3 Inconclusive: Regardless of the presence or absence of a line in the "T" (test) region, valid results will only be recorded if a pink line is present in the "C" (control) region. If no pink line is visible in this region, the test should be repeated if sufficient sample remains.

6.5 Limitations

6.5.1 False negative results may be obtained with the ABAcard® p30 test due to "High Dose Hook Effect". The high dose hook effect occurs when there are very high concentrations of p30 in a sample. If a high concentration of p30 is suspected, a 1:10 to 1:10,000 dilution of the sample can be created and retested to confirm the test result.

6.5.2 The results of this test should not be read after 10 minutes since non- specific reactions may occur and may result in false positives.

6.5.3 Prostate specific antigen has been reported in lower concentrations in other bodily fluids such as breast milk, amniotic fluid, and male urine.

6.5.4 Non-biological fluids such as feminine washes, energy drinks, and samples that alter the pH of the test have been shown to produce positive results.

6.6 Safety

6.6.1 Caution should be used when handling kit reagents. Personal protective equipment should be used at all times. Kit reagents contain sodium azide as a preservative which may react with lead or copper in plumbing. Upon disposal, always flush with large volumes of water to prevent build up.

6.7 References

6.7.1 ABAcards® p30 Validation, Metro Nashville Police Department Crime Laboratory Forensic Biology Unit, September 2014

6.7.2 ABAcards® p30 Non-biological Fluids Performance Check, Metro Nashville Police Department Crime Laboratory Forensic Biology Unit, September 2014

6.7.3 Abacus Diagnostics, Inc. ABAcard® p30 Test for The Forensic Identification of Semen: Technical Information Sheet. Updated 3/03.

6.7.4 Hobbs, Marcia M et al. Vaginal swab specimen processing methods influence performance of rapid semen detection tests: A cautionary tale. Contraception, September 2010, Vol. 82, No. 3: 291-295.

6.7.5 SERATEC. PSA in Body Fluids: An Overview for Users of the SERATEC PSA SEMIQUANT Tests.

6.7.6 DiFrancesco, J. Re-evaluation Seratec PSA Semiquant Test at the USACIL. AFFS presentation slides.

7. Sample Preparation

7.1 Removal of Hair or Tissue from Mounted Slides

7.1.1 Scope

7.1.1.1 To describe the process of removing hair or tissue from mounted slides.

7.1.2 Equipment/Materials/Reagents

7.1.2.1 Forceps

7.1.2.2 Scalpel/Scribe

7.1.2.3 Xylene

7.1.2.4 Ethanol

7.1.2.5 Ultrapure water

7.1.2.6 1.5ml Microcentrifuge tube

7.1.3 Procedure

7.1.3.1 A scribe may be used to score the coverslip around a hair root portion to be removed, and either process below can be used to remove the scored section of coverslip.

7.1.3.2 The coverslip may be removed by carefully pipetting xylene around the edges of the coverslip. If the coverslip does not loosen and come off, the entire slide can be covered with xylene for one or more hours until the coverslip has loosened.

7.1.3.3 The coverslip may also be removed by freezing the slide in a -20°C freezer for at least 20 minutes, then prying the coverslip off with a scalpel.

7.1.3.4 Carefully remove the hair (or tissue), or scrape the semen smear, and place in a 1.5ml tube.

7.1.3.5 Wash in 500µL xylene to remove excess mounting medium.

7.1.3.6 Follow with a wash of 500µL ethanol and a final rinse of autoclaved ultrapure water.

7.1.4 Safety

7.1.4.1 Caution should be used when handling glass. Personal protective equipment should be worn at all times. Xylene is a chemical irritant and flammable; use caution when handling and avoid exposure to open flame. Use in a fume hood when possible.

7.1.5 References

7.1.5.1 Washington State Patrol Crime Laboratory. STR Analysis Procedures. Recovering Slide-Mounted Hairs or Semen Smears.

7.2 Whole Blood Specimens

7.2.1 Scope

7.2.1.1 To describe the process for preparation of whole blood liquid specimens for DNA testing.

7.2.2 Equipment/Materials/Reagents

7.2.2.1 Whole blood specimen in purple top vacutainer tube (PPT)

7.2.2.2 Bloodstain card

7.2.2.3 Decontaminant

7.2.2.4 Laboratory wipes

7.2.2.5 Ink pen or marker

7.2.2.6 Disposable pipette or pipette with filter tip

7.2.3 Procedure

7.2.3.1 Document the packaging, conditions, and any identifying information on the blood tube.

7.2.3.2 At minimum, label the bloodstain card with:

7.2.3.2.1 Name of source

7.2.3.2.2 Incident or Lab number

7.2.3.2.3 Item number

7.2.3.2.4 Date card was spotted

7.2.3.2.5 Preparer's initials

7.2.3.3 Mix the blood by gently inverting the tube several times.

7.2.3.3.1 CAUTION! Ensure the stopper does not dislodge from the top of the tube.

7.2.3.4 Use a wipe to uncap the blood tube.

7.2.3.5 Use a disposable pipette, or pipette with filter tip, to transfer the blood from the tube to the appropriately labeled bloodstain card. Discard any excess blood remaining in the tube.

7.2.3.5.1 CAUTION! Do not over-saturate the card.

7.2.3.6 Allow the bloodstain card to **COMPLETELY AIR DRY** prior to packaging and sealing in an appropriately labeled package.

7.2.4 Safety

7.2.4.1 Perform the procedure in a well-ventilated area, preferably in the hood designated for the processing of known samples. If a barrier, such as a sash, does not separate the preparer from the blood, a face shield should be worn. Adhere to the general safety precautions outlined in Evidence Handling/ General Safety Section of the MNPD-CL Forensic Biology Quality Manual.

7.2.5 References

7.2.5.1 Evidence Handling/ General Safety Section of the MNPD-CL Forensic Biology Quality Manual

7.3 Liquid Samples, including oral rinses and condoms containing liquid

7.3.1 Scope

7.3.1.1 To describe the process for preparation of liquid samples for Serology and/or DNA testing.

7.3.2 Equipment/Materials/Reagents

7.3.2.1 Vortex

7.3.2.2 Decontaminant

7.3.2.3 Laboratory wipes

7.3.2.4 Sterile nylon flocked swab(s)

7.3.2.5 Sterile cotton swab(s) (if flocked swabs are unavailable)

7.3.3 Procedure

7.3.3.1 Document the packaging, conditions, and any identifying information on the item.

7.3.3.2 For oral rinses, centrifuge and /or allow cellular material to settle to the bottom of the tube or container. Discard the supernatant and collect the sediment on multiple swabs.

7.3.3.3 For condoms containing a liquid sample, preserve the liquid on multiple swabs.

7.3.3.4 If ample amount of sediment or liquid is present, collect on at least four swabs. If desired, pipette a portion of the sediment or liquid to a labeled microscope slide and proceed with slide preparation as outlined in Section 5.0 Microscopic Examination for Sperm.

7.3.3.5 Allow the swabs to **COMPLETELY AIR DRY** prior to packaging and sealing in an appropriately labeled package, or proceed using the process described in **Decision Tree for Semen/Sperm Screening Sexual Assault Kits** found in The Evidence Handling/Suspected Semen Section of the MNPD-CL Forensic Biology Quality Manual.

7.3.4 Safety

7.3.4.1 Adhere to the general safety precautions outlined in Evidence Handling/General Safety Section of the MNPD-CL Forensic Biology Quality Manual.

7.3.5 References

7.3.5.1 Virginia Department of Forensic Science. Forensic Biology Section, Screening and Collection for DNA Analysis. Semen Analysis.

7.4 Cutting Items for Re-extraction

7.4.1 Scope

7.4.1.1 To describe the process for re-cutting items for DNA re-extraction testing.

7.4.2 Equipment/Materials/Reagents

7.4.2.1 Scalpels/scissors/tweezers

7.4.2.2 Decontaminant

7.4.2.3 Laboratory wipes

7.4.2.4 Ink pen or marker

7.4.2.5 Sterile tube(s)

7.4.2.6 Re-extracted Sample Checklist

7.4.3 Procedure

7.4.3.1 Document notes on previously created serology sheet or on a new form.

7.4.3.1.1 Note: Additions to previously created serology sheet must be clear as to what actions were performed on what date.

7.4.3.2 Open the Re-extracted Sample Checklist.

7.4.3.3 Re-cut/swab the item and place in sterile labeled tube.

7.4.3.4 Once all steps have been completed, sign the analyst box on the Re-extracted Sample Checklist and include the form with the casefile.

8. Non-Differential Extraction Procedures

8.1 EZ1 Trace Protocol

8.1.1 Scope

8.1.1.1 To describe the process of robotic DNA extraction of questioned or known DNA samples using the EZ1 Advanced XL System.

8.1.2 Equipment/Materials/Reagents

8.1.2.1 EZ1 Advanced XL DNA Extraction Robot

8.1.2.2 EZ1 DNA Investigator Kit

8.1.2.3 G2 Buffer

8.1.2.4 Proteinase K

8.1.2.5 Thermomixer w/2.0mL block

8.1.2.6 Scissors

8.1.2.7 Lyse and Spin Basket Kit

8.1.2.8 Vortex

8.1.2.9 Centrifuge

8.1.2.10 Qiagen Pipette Tips

8.1.2.11 Qiagen Pipette Tip Holder

8.1.2.12 1.5mL Qiagen Elution Tubes

8.1.2.13 Autoclaved Water

8.1.2.14 Pipettes

8.1.3 Standards and Controls

8.1.3.1 See Reagents/Supplies of the FB Quality Manual for reagents that require QC prior to use.

8.1.3.2 At least one reagent blank will be run with each extraction batch. A reagent blank is used to test all of the reagents used throughout the DNA process for potential extraneous DNA. It is subjected to the same processes as the DNA samples; however, no DNA is present.

8.1.3.3 An extraction batch is defined as a group of samples which are subjected to the same processes at the same time on the same instrument and that are associated with the same extraction blank.

8.1.4 Calibration

8.1.4.1 The thermomixers shall be calibrated once per year to ensure they are achieving the proper temperature range.

8.1.4.2 The EZ1 instruments shall undergo annual preventative maintenance followed by a performance check to ensure the instruments are performing properly, prior to running casework samples.

8.1.4.3 Refer to The Maintenance and Calibration Protocols of the MNPD-CL Forensic Biology Quality Manual.

8.1.5 Procedure

8.1.5.1 Excise an appropriately sized sample and place it into a clean, labeled Lyse and Spin Basket tube.

8.1.5.2 Prepare a master mix of diluted G2 Buffer and Proteinase K. The mix is prepared using 480µL of the diluted G2 Buffer (1:2 dilution with water: 240µL of G2 Buffer and 240µL of Autoclaved Water) and 20µL Proteinase K per sample. The number of samples calculated may be increased to ensure enough master mix is prepared.

8.1.5.3 Aliquot 500µL of the master mix into each of the Lyse & Spin baskets.

8.1.5.4 Mix the sample by vortexing for 5-15 seconds.

8.1.5.5 Incubate the sample at 56°C for 1-2 hours. While incubating, shake the sample at 900 rpm.

8.1.5.6 Centrifuge the sample for 1 min at 10,000x RCF. Make sure all the liquid has passed through the membrane into the collection tube. Additional spin(s) may be necessary and can be carried out up to 20,000x RCF. After all liquid has passed through the membrane, using clean scissors, cut the hinge of the microcentrifuge tube (with the spin basket still in place and the lid closed).

8.1.5.7 Turn on the EZ1.

8.1.5.7.1 Load the EZ1 DNA Investigator Cartridge into the appropriate position on the EZ1 cartridge rack.

8.1.5.7.2 Load the Qiagen pipette tip and pipette tip holder into the appropriate position on row 2 of the deck of the EZ1.

8.1.5.7.3 Load a clean labeled Qiagen 1.5mL elution tube into the appropriate position on row 1 of the deck of the EZ1.

8.1.5.7.3.1 **NOTE**: If two extracts from the same sample are being combined, the previously labeled elution tube containing the original extract may be placed in the elution tube position.

8.1.5.7.4 Remove the lid and spin basket and discard them if necessary. Load the sample tube into the appropriate position on row 4 of the deck of the EZ1.

8.1.5.7.5 Run the Trace Protocol. Select to elute in TE buffer. Elute in 50, 100, or 200µL depending on sample type.

8.1.5.8 Start run.

8.1.6 Limitations

8.1.6.1 **CAUTION**: Certain porous materials interfere with bead extraction processes, preventing the recovery of DNA from a sample.

8.1.7 Safety

8.1.7.1 Buffers in the reagent cartridges contain guanidine salts, which can form highly reactive compounds when combined with bleach. Do not use bleach or allow bleach to contact reagent waste. Caution should be used when handling chemicals and personal protective equipment should be used at all times.

8.1.8 References

8.1.8.1 EZ1 DNA Investigator Handbook (2009). EZ1 DNA Investigator Kit. Qiagen, Hilden, Germany.

8.1.8.2 EZ1 Advanced XL User Manual (2009). EZ1 Advanced XL DNA Extraction Robot. Qiagen, Hilden, Germany.

8.1.8.3 DNA Extraction with QIAGEN EZ1® DNA Investigator Kit and EZ1® Advanced XL (July 2014), Sorenson Forensics at MNPD-CL Forensic Biology Unit.

8.1.8.4 Developmental Validation for QIAGEN EZ1® Robotic Platform and DNA Investigator® Silica-based Extraction Chemistry (October 2013). Qiagen.

8.1.8.5 EZ1® Validation Guide for the EZ1 DNA Investigator Kit, BioRobot® EZ1, EZ1 Advanced and EZ1 Advanced XL (June 2009). Qiagen.

8.1.8.6 Phillips, Kirsty, Nicola McCallum, and Lindsey Welch. "A Comparison of Methods for Forensic DNA Extraction: Chelex-1001 and TheQIAGEN DNA Investigator Kit (manual and Automated)." Forensic Science International: Genetics (2012): 282-85.

9. Differential Extraction Procedures

9.1 Automated Separation Method

9.1.1 Scope

9.1.1.1 To describe the process of extracting DNA from evidence samples suspected to contain semen utilizing an automated separation of the sperm and non-sperm fractions via the QIAcube.

9.1.2 Equipment/Materials/Reagents

9.1.2.1 EZ1 Advanced XL DNA Extraction Robot

9.1.2.2 EZ1 DNA Investigator Kit

9.1.2.3 QIAcube HID Differential Washing Station

9.1.2.4 Thermomixer

9.1.2.5 Scissors

9.1.2.6 Buffer ATL Working Solution

9.1.2.7 Proteinase K

9.1.2.8 Qiagen disposable 30mL reagent bottles

9.1.2.9 1000µL Qiagen wide-bore filter tips

9.1.2.10 Qiagen Rotor Adapter and Holder

9.1.2.11 1.5mL Qiagen sample tube

9.1.2.12 2.0mL Qiagen sample tube

9.1.2.13 Shaker Rack Plugs

9.1.2.14 Screw caps

9.1.2.15 G2 Buffer

9.1.2.16 Vortex

9.1.2.17 DTT

9.1.2.18 Autoclaved Water

9.1.2.19 Tweezers

9.1.2.20 Promega Spin Baskets

9.1.2.21 Centrifuge

9.1.2.22 Qiagen Pipette Tips

9.1.2.23 Qiagen Pipette Tip Holder

9.1.2.24 1.5mL Qiagen Elution Tubes

9.1.2.25 Pipettes

9.1.3 Standards and Controls

9.1.3.1 See Reagents/Supplies of the FB Quality Manual for reagents that require QC prior to use.

9.1.3.2 At least one reagent blank will be run with each extraction batch. A reagent blank is used to test all of the reagents used throughout the DNA process for potential extraneous DNA. It is subjected to the same processes as the DNA samples; however, no DNA is present.

9.1.3.3 An extraction batch is defined as a group of samples which are subjected to the same processes at the same time on the same instrument and that are associated with the same extraction blank.

9.1.4 Calibration

9.1.4.1 The thermomixers shall be calibrated once per year to ensure they are achieving the proper temperature range.

9.1.4.2 The EZ1 instruments shall undergo annual preventative maintenance followed by a performance check to ensure the instruments are performing properly, prior to running casework samples.

9.1.4.3 Refer to the Maintenance and Calibration Protocols of the MNPD-CL Forensic Biology Quality Manual.

9.1.4.4 The QIAcube instruments shall undergo annual preventative maintenance followed by a performance check to ensure the instruments are performing properly, prior to running casework samples.

9.1.5 Procedure

9.1.5.1 Place sample cutting or Copan swab head(s) into a clean, labeled 1.5mL Qiagen sample tube.

9.1.5.2 Prepare a master mix of diluted ATL and Proteinase K. The mix is prepared using 480µL of the diluted ATL and 20µL Proteinase K per sample. The number of samples calculated may be increased to ensure enough master mix is prepared.

9.1.5.3 Aliquot 500µL of the master mix into each of the extraction tubes.

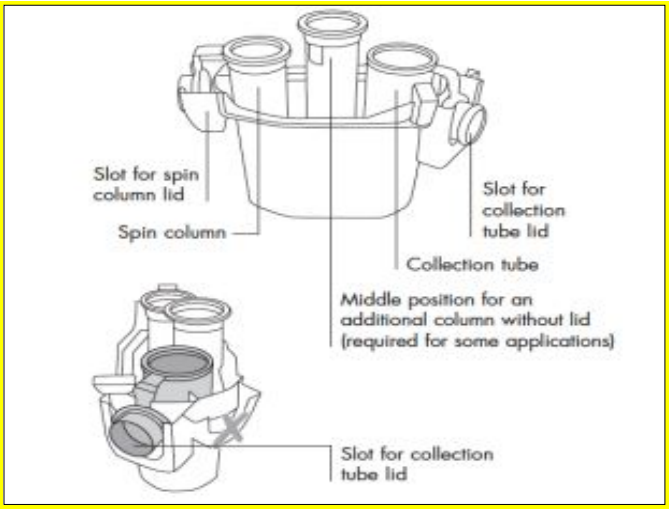
9.1.5.4 Mix the sample by vortexing for 5-15 seconds. Briefly centrifuge the sample to collect all the liquid into the tube.

9.1.5.5 Incubate the sample at 56°C for 1.5-2 hours. While incubating, shake the sample at 900rpm.

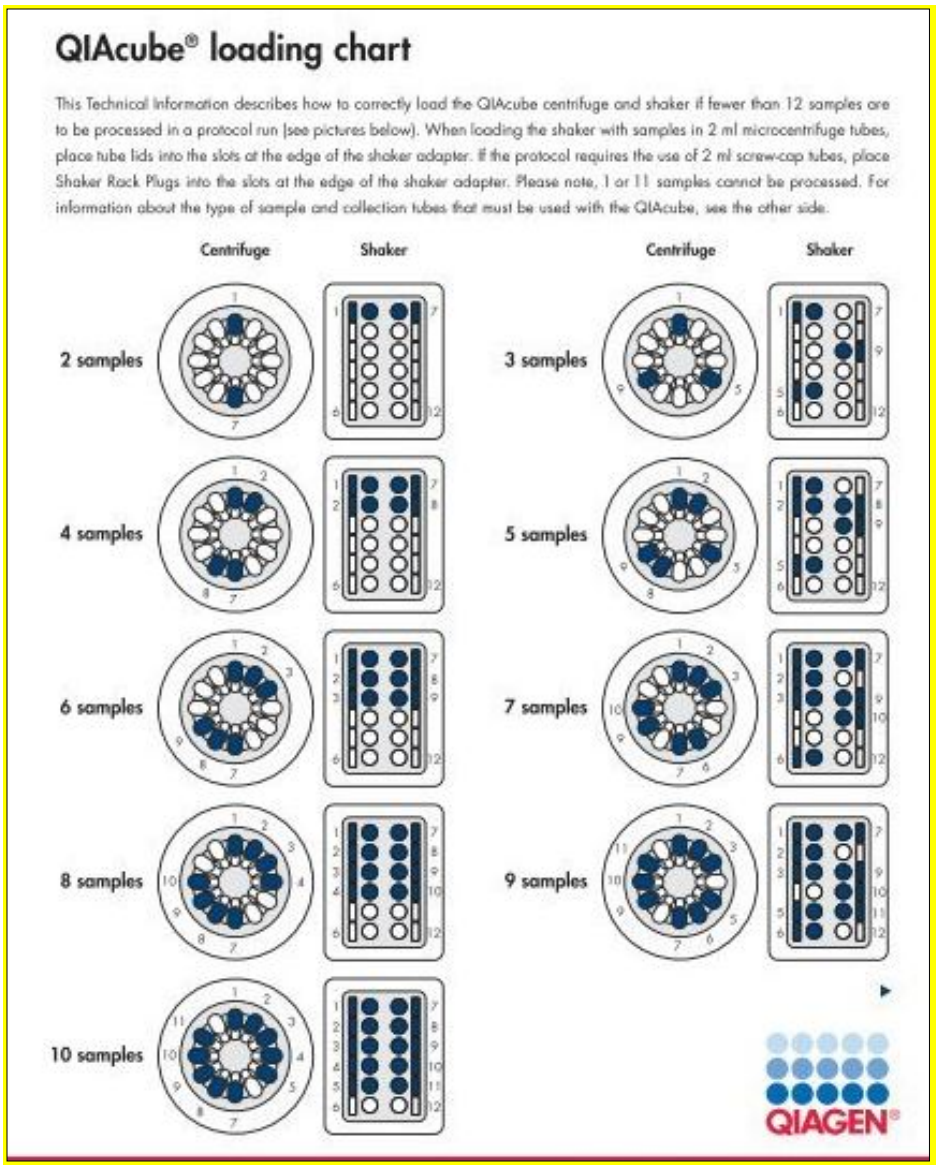
9.1.5.6 Turn on and clean the QIAcube instrument.

9.1.5.7 After incubation, pulse spin the sample to collect all the liquid. Place the cutting/swab from the sample into a clean spin basket using clean tweezers.Return the spin basket to the tube and centrifuge the basket and tube for 5 minutes at 10,000 x RCF. Discard the basket with the cutting/swab inside.

9.1.5.8 Place the 1.5mL Qiagen sample tube in position 3 (noted as collection tube in picture below) of the QIAcube rotor adapter.

9.1.5.8.1 

9.1.5.9 Place the rotor adapter in the proper position of the centrifuge. Refer to the QIAcube loading chart.



QIAcube® loading chart

This Technical Information describes how to correctly load the QIAcube centrifuge and shaker if fewer than 12 samples are to be processed in a protocol run (see pictures below). When loading the shaker with samples in 2 ml microcentrifuge tubes, place tube lids into the slots at the edge of the shaker adapter. If the protocol requires the use of 2 ml screw-cap tubes, place Shaker Rack Plugs into the slots at the edge of the shaker adapter. Please note, 1 or 11 samples cannot be processed. For information about the type of sample and collection tubes that must be used with the QIAcube, see the other side.

Centrifuge Shaker

2 samples

3 samples

4 samples

5 samples

6 samples

7 samples

8 samples

9 samples

10 samples

QIAGEN®

9.1.5.9.1

9.1.5.10 Place a clean, labeled 2.0mL Qiagen sample tube into the proper position on the shaker of the QIAcube. Refer to the QIAcube loading chart. Insert shaker rack plugs into the corresponding slots at the edges of the shaker rack.

9.1.5.11 Fill both tip racks of the QIAcube with 1000µL Qiagen wide-bore filter tips.

9.1.5.12 Place a 30mL reagent bottle with at least 2.0mL of G2 Buffer per sample into position 1 of the reagent bottle rack of the QIAcube.

9.1.5.13 Place a 30mL reagent bottle with at least 1.0mL of autoclaved water per sample into position 2 of the reagent bottle rack of the QIAcube.

9.1.5.14 Close the QIAcube lid and start the “Separate and Lyse 12A Mod” Protocol on the QIAcube. To start the protocol, select “DNA” > select “Pipetting” > select “Epithelial and

Sperm Cell" > select "Separate and Lyse 12A Mod". Then, follow prompts to begin the run by selecting "Start".

9.1.5.15 When the protocol is finished, remove the 2.0mL Qiagen sample tube from the shaker. This is the non-sperm (epithelial) fraction. The non-sperm fraction can then be processed following the steps outlined in section 8.1.5 of the MNPD-CL Forensic Biology Technical Procedures Manual, beginning with turning on the EZ1.

9.1.5.16 Refill the tip racks with 1000µL Qiagen wide-bore filter tips and start the "Separate and Lyse 12B Mod" Protocol on the QIAcube by following the above steps. When the protocol is finished, remove the 1.5 mL Qiagen sample tube from position 3 of the QIAcube rotor adapter. Wipe the outside of the tubes with 10% bleach then 70% ethanol. This is the sperm fraction.

9.1.5.17 Prepare a master mix for the sperm fraction of G2 Buffer, Proteinase K, and DTT. The mix is prepared using 160µL G2 Buffer, 10µL Proteinase K, and 40µL DTT per sample. The number of samples calculated may be increased to ensure enough master mix is prepared.

9.1.5.18 Aliquot 210µL of the master mix into each of the sperm fraction tubes.

9.1.5.19 Mix the sample by vortexing for 5-15 seconds. Briefly centrifuge the sample to collect all the liquid into the tube.

9.1.5.20 Incubate the sample at 70°C for 10 min while shaking at 900rpm. After incubation, vortex the sample for 10 seconds, pulse spin to collect the liquid, and remove the lid by cutting the hinge using clean scissors.

9.1.5.21 The sperm fraction can then be processed following the steps outlined in Section 8.1.5 of the MNPD-CL Forensic Biology Technical Procedures Manual, beginning with turning on the EZ1.

9.1.6 Limitations

9.1.6.1 This procedure is not suitable for extraction of DNA from samples in which no semen is suspected. For extracting DNA from samples in which no semen is suspected, refer to Section 8.0 of the MNPD-CL Forensic Biology Technical Procedures Manual.

9.1.7 Safety

9.1.7.1 Buffers in the reagent cartridges contain guanidine salts, which can form highly reactive compounds when combined with bleach. Do not use bleach or allow bleach to contact

reagent waste. Caution should be used when handling chemicals and personal protective equipment should be used at all times.

9.1.8 References

9.1.8.1 Developmental Validation of QIAcubeTM Automated Platform and Differential Wash Protocol for Forensic DNA Testing Laboratories (October 2013). Qiagen.

9.1.8.2 EZ1 DNA Investigator Handbook (2009). EZ1 DNA Investigator Kit. Qiagen, Hilden, Germany.

9.1.8.3 EZ1 DNA Investigator Handbook (July 2014). EZ1 DNA Investigator Kit. Qiagen, Hilden, Germany.

9.1.8.4 EZ1 Advanced XL User Manual (2009). EZ1 Advanced XL DNA Extraction Robot. Qiagen, Hilden, Germany.

9.1.8.5 QIAcube User Manual (2008). QIAcube HID Differential Washing Station. Qiagen, Hilden, Germany.

9.1.8.6 Differential Processing of Semen Samples using the QIAcube® Differential Washing Station Validation Report (August 2014), Sorenson Forensics at MNPD-CL Forensic Biology Unit.

9.2 Manual Separation Method

9.2.1 Scope

9.2.1.1 To describe the process of extracting DNA from semen stains utilizing a manual separation of the sperm and non-sperm fractions. This method is intended for use only if the QIAcube is unavailable for use.

9.2.2 Equipment/Materials/Reagents

9.2.2.1 EZ1 Advanced XL DNA Extraction Robot

9.2.2.2 EZ1 DNA Investigator Kit

9.2.2.3 Thermomixer

9.2.2.4 Scissors

9.2.2.5 Buffer ATL Working Solution

9.2.2.6 Proteinase K

9.2.2.7 G2 Buffer

9.2.2.8 DTT

9.2.2.9 Autoclaved Water

9.2.2.10 Promega Spin Basket

9.2.2.11 1.5mL Qiagen Sample Tube

9.2.2.12 Tweezers

9.2.2.13 Vortex

9.2.2.14 Centrifuge

9.2.2.15 Pipettes

9.2.3 Standards and Controls

9.2.3.1 See Reagents/Supplies of the FB Quality Manual for reagents that require QC prior to use.

9.2.3.2 At least one reagent blank will be run with each extraction batch. A reagent blank is used to test all of the reagents used throughout the DNA process for potential extraneous DNA. It is subjected to the same processes as the DNA samples; however, no DNA is present.

9.2.3.3 An extraction batch is defined as a group of samples which are subjected to the same processes at the same time on the same instrument and that are associated with the same extraction blank.

9.2.4 Calibration

9.2.4.1 The thermomixers shall be calibrated once per year to ensure they are achieving the proper temperature range.

9.2.4.2 The EZ1 instruments shall undergo annual preventative maintenance followed by a performance check to ensure the instruments are performing properly, prior to running casework samples.

9.2.4.3 Refer to the Maintenance and Calibration Protocols of the MNPD-CL Forensic Biology Quality Manual.

9.2.5 Procedure

9.2.5.1 Place sample cutting or Copan swab head(s) into a clean, labeled 1.5mL Qiagen sample tube.

9.2.5.2 Prepare a master mix of diluted Buffer ATL Working Solution and Proteinase K. The mix is prepared using 480µL of the diluted ATL Buffer and 20µL Proteinase K per sample. The number of samples calculated may be increased to ensure enough master mix is prepared.

9.2.5.3 Aliquot 500µL of the master mix into each of the extraction tubes.

9.2.5.4 Mix the sample by vortexing for 5-15 seconds. Briefly centrifuge the sample to collect all the liquid into the tube.

9.2.5.5 Incubate the sample at 56°C for 1.5-2 hours. While incubating, shake the sample at 900 rpm.

9.2.5.6 After incubation, pulse spin the sample to collect all the liquid. Place the cutting/swab from the sample in a clean spin basket. Centrifuge basket and tube for 5 minutes at 10,000x RCF. Discard the basket with the sample cutting/swab inside.

9.2.5.7 Centrifuge the sample for 5 minutes at 10,000x RCF. Transfer the supernatant to a new clean, labeled 1.5mL microcentrifuge tube. This is the non-sperm (epithelial) fraction.

9.2.5.8 The non-sperm fraction can then be processed following the steps outlined in section 8.1.5 of the MNPD-CL Forensic Biology Technical Procedures Manual, beginning with turning on the EZ1.

9.2.5.9 Add 500µL G2 Buffer to the pellet.

9.2.5.10 Mix the sample by vortexing for 5-15 seconds. Centrifuge the sample for 5 minutes at 10,000x RCF.

9.2.5.11 Carefully remove the supernatant (without disturbing the pellet) and discard it.

9.2.5.12 Repeat the wash step with G2 Buffer two additional times.

9.2.5.13 Add 700µL autoclaved water to the sample.

9.2.5.14 Mix the sample by vortexing for 5-15 seconds. Centrifuge the sample for 5 minutes at 10,000x RCF.

9.2.5.15 Remove all the supernatant except approximately 50uL (without disturbing the pellet) and discard it. This is the sperm fraction.

9.2.5.16 Prepare a master mix for the sperm fraction of G2 Buffer, Proteinase K, and DTT. The mix is prepared using 160µL G2 Buffer, 10µL Proteinase K, and 40µL DTT per sample. The number of samples calculated may be increased to ensure enough master mix is prepared.

9.2.5.17 Aliquot 210µL of the master mix into each of the sperm fraction tubes.

9.2.5.18 Mix the sample by vortexing for 5-15 seconds. Briefly centrifuge the sample to collect all the liquid into the tube.

9.2.5.19 Incubate the sample at 70°C for 10 min while shaking at 900rpm. After incubation, vortex the sample for 10 seconds, pulse spin to collect the liquid, and remove the lid by cutting the hinge using clean scissors.

9.2.5.20 The sperm fraction can then be processed following the steps outlined in section 8.1.5 of the MNPD-CL Forensic Biology Technical Procedures Manual, beginning with turning on the EZ1.

9.2.6 Limitations

9.2.6.1 This procedure is not suitable for extraction of DNA from samples in which no semen is suspected. For extracting DNA from samples in which no semen is suspected, refer to Section 8.0 of the MNPD-CL Forensic Biology Technical Procedures Manual.

9.2.7 Safety

9.2.7.1 Buffers in the reagent cartridges contain guanidine salts, which can form highly reactive compounds when combined with bleach. Do not use bleach or allow bleach to contact reagent waste.

9.2.7.2 **CAUTION** should be used when handling chemicals and personal protective equipment should be used at all times.

9.2.8 References

9.2.8.1 EZ1 DNA Investigator Handbook (2009). EZ1 DNA Investigator Kit. Qiagen, Hilden, Germany.

9.2.8.2 EZ1 DNA Investigator Handbook (July 2014). EZ1 DNA Investigator Kit. Qiagen, Hilden, Germany.

9.2.8.3 EZ1 Advanced XL User Manual (2009). EZ1 Advanced XL DNA Extraction Robot. Qiagen, Hilden, Germany.

9.2.8.4 Differential Processing of Semen Samples using the QIAcube Differential Washing Station Validation Report (August 2014), Sorenson Forensics at MNPD-CL Forensic Biology Unit.

10. Quantitation

10.1 Setup Protocol

10.1.1 Scope

10.1.1.1 To describe the manual preparation of DNA samples and standards for quantitation using the Plexor® HY System.

10.1.2 Equipment/Materials/Reagents

10.1.2.1 Plexor HY System Kit

10.1.2.1.1 Plexor® HY 2X Master Mix

10.1.2.1.2 Plexor® HY 20X Primer/IPC Mix

10.1.2.1.3 Plexor® HY Male Genomic DNA Standard, 50ng/µl

10.1.2.1.4 Water, Amplification Grade

10.1.2.2 TE Buffer

10.1.2.3 1.5mL Microcentrifuge tubes

10.1.2.4 1.5 mL low adhesion tubes

10.1.2.5 96-well optical grade PCR plate

10.1.2.6 Optical adhesive cover and plate cover applicator

10.1.2.7 Optical Support Base

10.1.2.8 7500 Real-Time PCR System

10.1.2.9 Qiagility

10.1.2.10 Qiagility Software v4.18.1

10.1.2.11 Vortex

10.1.2.12 Centrifuge

10.1.2.13 Pipettes

10.1.3 Standards and Controls

10.1.3.1 A standard curve is generated using DNA standards in the range of 3.2pg/µl to 50ng/µl. Plexor HY Male Genomic DNA Standard at an undiluted concentration of 50ng/µl is used to prepare a serial dilution of the remaining DNA standards. Each standard point will be run in duplicate.

10.1.3.2 Creating the standards:

10.1.3.2.1 Upon first use of each standard tube, thaw and vortex the Plexor HY Male Genomic DNA Standard at high speed for 10sec. Do not centrifuge standard after vortexing.

10.1.3.2.2 Prepare serial dilutions for the Plexor HY standards as indicated below using 1.5mL low adhesion tubes. Vortex each dilution for 10sec before removing an aliquot. Do not centrifuge standards after vortexing. It is recommended to change gloves after handling first two concentration points of standards.

Standard	Concentration	Volume of DNA	Volume of TE
Standard 1	50ng/ul	Use neat standard	0ul
Standard 2	10ng/ul	10µl of neat standard	40ul
Standard 3	2ng/ul	10µl of 10ng/µl dilution	40ul
Standard 4	0.4ng/ul	10µl of 2ng/µl dilution	40ul
Standard 5	0.08ng/ul	10µl of 0.4ng/µl dilution	40ul
Standard 6	0.016ng/ul	10µl of 0.08ng/µl dilution	40ul
Standard 7	0.0032ng/ul	10µl of 0.016ng/µl dilution	40ul

10.1.3.2.3 Fresh standards should be made each day and stored at 2-8°C when not in use. Do not refreeze DNA standards.

10.1.4 Calibration

10.1.4.1 Each 7500 shall undergo annual preventative maintenance followed by a performance check to ensure the instruments are performing properly, prior to running casework samples.

10.1.4.2 Refer to the Maintenance and Calibration Protocols of the MNPD-CL Forensic Biology Quality Manual.

10.1.5 Procedure

10.1.5.1 Thaw Plexor HY kit components at room temperature.

10.1.5.2 Vortex Master Mix and Primer Mix for 10sec. Tap tube to remove any reaction mix from the top of the tube. Do not centrifuge kit components or reaction mix after vortexing.

10.1.5.3 Determine the number of reactions to be set up, including standards. Also, add enough for at least 4 additional reactions.

10.1.5.4 Prepare reaction mix:

Component	Volume (Per Reaction)
Plexor HY 2X Master Mix	10µl
Water, Amplification Grade	7µl
Plexor HY 20X Primer/IPC Mix	1µl
FINAL VOLUME	18µl

10.1.5.5 Proceed to the Manual Plating or QIAgility Plating quantitation procedure.

10.1.5.6 Manual Plating

10.1.5.6.1 Vortex prepared reaction mix for 10secs. Tap tube to remove any reaction mix from the top of the tube.

10.1.5.6.2 Add 18µl reaction mix to each well of an optical-grade PCR plate as determined by the plate setup sheet. The reaction plate should be seated in an optical support base and should not be touching the bottom of the wells.

10.1.5.6.3 Following the addition of master mix and prior to the addition of sample, check to ensure that master mix has been added to all appropriate wells.

10.1.5.6.4 Vortex and spin down samples

10.1.5.6.5 Add 2µl of DNA standards or unknown samples to reaction mix as determined by the plate setup sheet. DNA standards must be run in duplicate.

10.1.5.6.6 Seal plate with optical adhesive cover using plate cover applicator.

10.1.5.6.7 Centrifuge plate briefly to collect contents at bottom of wells and eliminate bubbles.

10.1.5.6.8 Proceed to Section 10.2 7500 Instrument Setup.

10.1.5.7 *QIAgility Plating*

10.1.5.7.1 Preparation Using the DNA Batch Workbook

10.1.5.7.1.1 Upon entering sample information into the DNA Batch Workbook, the “Quant” tab will populate with samples entered into the “Master List” tab. Save the information in the

"Quant QIA" tab as a .txt file (tab delimited). (Instruction will be listed at the top of the excel sheet in this tab.)

10.1.5.7.1.2 Reagent volumes needed for the quantitation set up will be calculated and listed in the Quant Set-up of the DNA Batch Workbook. (Note: add three additional samples to the ones listed on the sheet. Do this by leaving "0" in three of the remaining wells. Remove the "0"'s from any unused wells.)

10.1.5.7.2 QIAgility Instructions

10.1.5.7.2.1 Vortex prepared reaction mix for 10secs. Tap tube to remove any reaction mix from the top of the tube.

10.1.5.7.2.2 Turn on computer.

10.1.5.7.2.3 Turn on instrument.

10.1.5.7.2.4 Launch Qiagility Software v4.18.1. The QIAgility instrument lid must be closed for the software and hardware to initialize upon start-up and for a run to proceed.

10.1.5.7.2.5 Place prepared Plexor HY Reaction Mix into position G on the Reagent Block (R1) of the QIAgility. Using prepared quantitation standards, load the standards into positions I through O on the Reagent Block (R1).

10.1.5.7.2.6 Load samples tubes into the 4 x 8 well sample racks in the Sample Block (A2).

10.1.5.7.2.7 Place a 96 well optical plate in Reaction Block (C1).

10.1.5.7.2.8 Open the Quant protocol by going to "File" at the top left of the software screen, click "Open," and find the Quant protocol.

10.1.5.7.2.9 In the software, select the sample rack position A2. Click on the "Import" button and import the .txt file generated for your run.

10.1.5.7.2.10 Click on the green start arrow on the toolbar or select "Control/Start".

10.1.5.7.2.11 Check the pre-run report to verify the location and amount/volume of consumables and liquids that are required on the worktable for completion of the loaded run file.

10.1.5.7.2.12 The pre-run "Checklist" dialog box will appear. If no warnings or errors are listed, select the boxes to continue and click "OK" to start the run. If errors are listed, user intervention is required at this step.

10.1.5.7.2.12.1 **NOTE**: Care must be taken when opening the QIAgility lid during a run. It takes up to 10 seconds for the instrument to complete its current movement and for the pause to take effect.

10.1.5.7.2.13 Follow the prompted action pop-up windows during the duration of the Quant protocol.

10.1.5.7.2.14 Upon completion of the run, a "Post-run report" will appear. Save the "Post-run report" for the quant set up for the casefile.

10.1.5.7.2.15 Remove the optical grade PCR reaction plate from the QIAgility deck. Proceed to Section 10.2 7500 Instrument.

10.1.5.7.2.16 Seal plate with optical adhesive cover using plate cover applicator.

10.1.5.7.2.17 Centrifuge plate briefly to collect contents at bottom of wells and eliminate bubbles.

10.1.6 Limitations

10.1.6.1 Plexor HY Male Genomic DNA Standard may form aggregates or concentration gradients when frozen, which may result in sampling error. Ensure the standard is thawed at room temperature and vortex prior to use.

10.1.6.2 It is critical that the same reaction mix is used for the entire run. Ensure enough reaction mix is prepared to account for pipetting error.

10.1.6.3 Protect the reaction plate from extended light exposure or elevated temperature before cycling.

10.1.7 Safety

10.1.7.1 Caution should be used when handling chemicals and personal protective equipment should be used at all times.

10.1.7.2 The robotic arm of the Qiagility instrument moves during position calibration while the instrument lid is raised. Never click any buttons while parts of your body are within the instrument workspace.

10.1.8 References

10.1.8.1 Applied Biosystems. 2006. Validation Using SDS Software Version 1.2.3 on the Applied Biosystems 7500 Real-Time PCR System and the ABI PRISM® 7000 Sequence Detection System: User Bulletin.

10.1.8.2 Krenke, Benjamin E., et al. Developmental Validation of a Real-Time PCR Assay for the Simultaneous Quantification of Total Human and Male DNA: Developmental Validation of the Plexor® HY System. Promega Corporation, 2007.

10.1.8.3 Plexor HY Technical Manual (May 2013). Plexor® HY System for the Applied Biosystems 7500 and 7500 FAST Real-Time PCR Systems. Promeg, USA.

10.1.8.4 Internal Validation with Plexor® HY and 7500 Real-Time PCR System: (October 2014), Sorenson Forensics at MNPD-CL Forensic Biology Unit.

10.1.8.5 Internal Validation with QIAgility: : (2015), Sorenson Forensics at MNPD-CL Forensic Biology Unit.

10.1.8.6 QIAgility® User's Manual (June 2013). QIAGEN.

10.1.8.7 Washington State Patrol Casework STR Analysis Procedures Crime Laboratory Division. August 2013.

10.2 7500 Instrument Setup

10.2.1 Scope

10.2.1.1 To describe the process of setting up the Applied Biosystems 7500 instrument software and creating a template.

10.2.2 Equipment/Materials/Reagents

10.2.2.1 Applied Biosystems 7500

10.2.2.2 HID Real-Time PCR Analysis Software v1.2

10.2.2.3 Centrifuge

10.2.2.4 Optical Adhesive Cover

10.2.2.5 Plate Cover Applicator

10.2.3 Procedure

10.2.3.1 Turn on the 7500 instrument.

10.2.3.2 Open the HID software. Login with you initials or login as guest.

10.2.3.3 Open the 7500 instrument and place the plate with the well A1 in the upper left corner.

10.2.3.4 Select "Custom Assays" button, then file > new experiment > from template.

10.2.3.5 Import the Plexor HY template:

10.2.3.5.1 This PC > OS (C:) > Applied Biosystems > 7500 > Config > Templates > Plexor HY

10.2.3.6 Change the experiment name to match your Quant plate name.

10.2.3.7 Under the "Setup" > "Plate Set Up" heading:

10.2.3.7.1 Select the "Assign Targets and Samples" tab

10.2.3.7.1.1 Highlight wells containing DNA.

10.2.3.7.1.2 Select all targets (auto, IPC, PassRef, Y)

10.2.3.8 Start run > click "ok" > save to experiments folder on C: drive.

10.2.3.9 After the run is completed, remove the plate and discard it in the biohazard waste.

10.2.3.10 Export run data following the steps outlined in section 10.3.3. Once data is exported, turn off the instrument and close software.

10.2.3.11 For data analysis and data interpretation, see sections 10.4 and 10.5 respectively.

10.2.3.12 If, for some reason, the plate was loaded backwards (well A1 in lower right corner) or a well was missed when assigning targets, the run does not need to be redone. Return to the "Assign Targets and Samples" tab under the "Setup" > "Plate Set Up" heading. Clear all incorrectly assigned wells and associated targets. Then, select correct wells that contained DNA and subsequently assign targets.

10.2.3.12.1 IMPORTANT: Reanalyze the run by selecting the green "Analyze" button under the "Analysis" heading. Save the run and continue exporting the run data by following the steps outlined in section 10.3.3.

10.2.4 References

10.2.4.1 Applied Biosystems. 2006. Validation Using SDS Software Version 1.2.3 on the Applied Biosystems 7500 Real-Time PCR System and the ABI PRISM® 7000 Sequence Detection System: User Bulletin.

10.2.4.2 Krenke, Benjamin E., et al. Developmental Validation of a Real-Time PCR Assay for the Simultaneous Quantification of Total Human and Male DNA: Developmental Validation of the Plexor® HY System. Promega Corporation, 2007.

10.2.4.3 Plexor HY Technical Manual (May 2013). Plexor® HY System for the Applied Biosystems 7500 and 7500 FAST Real-Time PCR Systems. Promega, USA.

10.2.4.4 Internal Validation with Plexor® HY and 7500 Real-Time PCR System: (October 2014), Sorenson Forensics at MNPD-CL Forensic Biology Unit.

10.3 7500 Instrument Data Export into the Plexor® Analysis Software

10.3.1 Scope

10.3.1.1 To describe the process of exporting the data generated by the Applied Biosystems 7500 instrument into the Plexor Analysis Software.

10.3.2 Equipment/Materials/Reagents

10.3.2.1 Applied Biosystems 7500

10.3.2.2 HID Real-Time PCR Analysis Software v1.3

10.3.2.3 Plexor® Analysis Software (forensic release) v1.5.6.7

10.3.3 Procedure

10.3.3.1 Export from HID Software v1.3:

10.3.3.1.1 Click Analyze on the 7500 software. Then select "Export" from the top toolbar.

10.3.3.1.2 In the "Export Data" window, select the following "Export Properties":

10.3.3.1.2.1 Ensure that "Multicomponent Data" is selected.

10.3.3.1.2.2 File Type: Select .xls.

10.3.3.1.2.3 Specify the appropriate export file name & file location.

10.3.3.1.2.3.1 G drive (G:) > Instrument-DNA > Run Files > 7500 Real Time PCR > *Name of Instrument* > Year > Month

10.3.3.1.2.4 Select "Start Export".

10.3.3.1.3 Close HID Software and turn off 7500 instrument.

10.3.3.2 Import into Plexor Analysis Software v1.5.6.7:

10.3.3.2.1 Launch Plexor Analysis Software v1.5.6.7.

10.3.3.2.2 In the File menu, ensure "Set Passive Reference On Import" is selected. With HID software, the IC5 passive reference data is imported separately, and the normalization is applied within Plexor® Analysis Software.

10.3.3.2.3 In the File menu, select "Import New Run" or select the icon. 

10.3.3.2.4 Ensure "Applied Biosystems 7500 HID" is selected as the instrument type.

10.3.3.2.5 Ensure the target names and each of the dyes are assigned as follows:

10.3.3.2.5.1Autosomal (FL) – Amp & Melt boxes selected

10.3.3.2.5.2Y(CO560) – Amp & Melt boxes selected

10.3.3.2.5.3IPC (CR610) – Amp & Melt boxes selected

10.3.3.2.5.4 Passive Reference (IC5) – Pass. Ref box is selected (this is the only box selected)

10.3.3.2.6 Select "Next".

10.3.3.2.7 Enter information specific to your run in the Run Info screen, Step 2. "Operator Name" is required. Details such as the date, notes, title, name of the person performing the experiment, etc., also can be entered. These fields are optional.

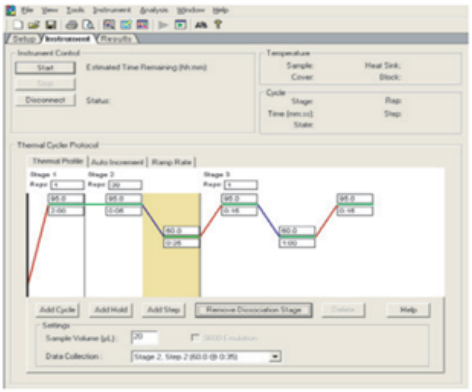
10.3.3.2.8 Select "Next".

10.3.3.2.9 In Step 3, use the File Import screen to specify the appropriate HID data file.

10.3.3.2.10 Ensure the number of amplification cycles is set to 38 and the range of melt temperature is set to 60 to 95.

10.3.3.2.11 Ensure the cycling parameters noted below are used when running samples on the 7500 RT PCR instrument utilizing Plexor HY.

Step	Temperature	Time	Number of Cycles
Initial denaturation:	95°C	2 minutes	1 cycle
Denaturation:	95°C	5 seconds	38 cycles
Annealing and extension:	60°C	35 seconds	
Melt temperature curve:	Use the default "Dissociation Function" settings.		



10.3.3.2.11.1

10.3.3.2.12 In the Advanced Options box, ensure that the Plexor Run Template is selected under Run Template. If not, click Edit, then Import and navigate to the Template located in the Instruments-Equipment Software folder of the Forensic Biology Unit G:\Instrument-DNA\Run Files\Plexor software and template.

10.3.3.2.13 Select "Finish" to complete the data import and open the Analysis Desktop.

10.3.4 References

10.3.4.1 Applied Biosystems. 2006. Validation Using SDS Software Version 1.2.3 on the Applied Biosystems 7500 Real-Time PCR System and the ABI PRISM® 7000 Sequence Detection System: User Bulletin.

10.3.4.2 Krenke, Benjamin E., et al. Developmental Validation of a Real-Time PCR Assay for the Simultaneous Quantification of Total Human and Male DNA: Developmental Validation of the Plexor® HY System. Promega Corporation, 2007.

10.3.4.3 Plexor HY Technical Manual (May 2013). Plexor® HY System for the Applied Biosystems 7500 and 7500 FAST Real-Time PCR Systems. Promeg, USA.

10.3.4.4 Internal Validation with Plexor® HY and 7500 Real-Time PCR System: (October 2014), Sorenson Forensics at MNPD-CL Forensic Biology Unit.

10.4 Plexor® HY and 7500 Instrument Data Analysis

10.4.1 Scope

10.4.1.1 To describe the analysis process and interpretation of the data by the Plexor® Analysis Software v1.5.6.7.

10.4.2 Equipment/Materials/Reagents

10.4.2.1 Plexor® Analysis Software (forensic release) v1.5.6.7

10.4.3 Procedure

10.4.3.1 After data import is complete, the PCR curves tab is displayed in the Analysis Desktop.

10.4.3.2 If the Plexor Run Template was not selected on import, the DNA standards must be defined:

10.4.3.2.1 Using the well selector, highlight wells that contain the DNA standards

10.4.3.2.2 Select Create Dilution Series icon.

10.4.3.2.3 Confirm that the series selected is a “Vertical Series” and the series is “Decreasing”. Enter 50 for the starting concentration and 5 for the dilution factor.

10.4.3.3 <u>Assign or edit sample names:</u>

10.4.3.3.1 Select the Sample IDs tab, select each well and enter the desired sample name. Repeat to enter the sample names for the other wells.

10.4.3.3.2 Samples with the same name will have their DNA quantities averaged in the Forensics Report created by the software.

10.4.3.3.2.1 **NOTE**: Samples cannot be exported from the forensics report that have the same name.

10.4.3.4 Alternatively, sample names may be copied from a Microsoft Excel spreadsheet. Highlight the sample names on the spreadsheet, select Copy. In the Edit menu of the Plexor Analysis Software, select Paste Sample IDs from Template. If copying sample names, the layout of the sample names in the spreadsheet must be the same as the layout of the samples within the PCR plate.

10.4.3.5 Once complete, click "Accept Changes".

10.4.3.6 Adjust the Expected Target Melt Temperature:

10.4.3.7 Select the PCR Curves tab

10.4.3.8 Select all wells that contain the DNA standards.

10.4.3.9 The Tm for each selected sample will be displayed. The expected melt temperature range must be adjusted for each dye. The average expected target melt temperatures are as follows:

10.4.3.9.1 FL 79-81°C

10.4.3.9.2 CO560 81-83°C

10.4.3.9.3 CR610 79-81°C

10.4.3.10 For some samples the IPC (CR610) may fall outside of this range by as much as 2°C. The amplification data, the Cq values in particular, are the primary means of analyzing the IPC data.

10.4.3.11 In the melt curves window of FL tab, drag the expected target melt temperature line to the midpoint of the melt curves. Alternatively, double-click on the line, and enter the desired temperature.

10.4.3.12 Repeat for CO560 and CR610 tabs.

10.4.3.13 Generating a Standard Curve:

10.4.3.13.1 In the autosomal channel (FL tab), select all samples and DNA standards.

10.4.3.13.2 Select "Add Standard Curve" to generate a standard curve and determine DNA concentrations of the unknowns based on the standard curve.

10.4.3.13.3 Repeat this for the Y channel (CO560 tab)

10.4.3.13.4 Select the Standard Curves tab to view the standard curves. The slope, Y-intercept, R2 value, and efficiency are displayed on the graph along with the samples that do not have a valid Cq value (meaning they did not cross the amplification threshold).

10.4.3.14 Run Quality Determination:

10.4.3.14.1 The standard curve for the autosomal target (FL) has an average slope in the range of -3.2 to -4.0 (rounding allowed).

10.4.3.14.2 The standard curve for the Y-chromosomal target (CO560) has an average slope in the range of -3.0 to -3.6 (rounding allowed).

10.4.3.14.3 The R2 value will be evaluated and must be ≥ 0.99.

10.4.3.14.4 If the slope value is outside of allowable autosomal and/or Y-chromosomal range and/or the R2 values <0.99, standards can be omitted as follows:

10.4.3.14.4.1 The sample type for the 0.0032ng/ µl standard point (one or both replicates) may be changed from standard to unknown. This will automatically be incorporated into the standard curves and may improve the slope value(s) and/or R2 value.

10.4.3.14.4.1.1 One additional replicate point may be changed from standard to unknown to achieve the appropriate slope value(s) and/or R2 value.

10.4.3.14.4.2 If the appropriate slope value(s) and/or R2 value cannot be achieved, quantitation of the samples must be repeated.

10.4.3.14.4.3 To remove a point from the standard curve

10.4.3.14.4.3.1 PCR Curves

10.4.3.14.4.3.1.1 Click the standard in the bottom of the screen that you would like removed.

10.4.3.14.4.3.1.2 Select the UNK symbol at the top of the page.

10.4.3.14.5 If the y-intercept changes significantly from run to run without a change in the slope value(s) or R2 value of the standard curve, then this suggests the DNA standard was not sufficiently mixed before use or has degraded.

10.4.3.15 <u>Generating a Forensic Report:</u>

10.4.3.15.1 Navigate to the “Forensics” tab at the top of the Plexor Analysis Software and select “Set Normalization and IPC Parameters”. No changes are necessary to the resulting pop-up window. Select “OK”.

10.4.3.15.2 A new tab labeled “Forensics” will populate under the “Reports” tab for your data. This Forensics report contains the data that will be used downstream.

10.4.3.15.3 Under reports > forensics

10.4.3.15.3.1 Click the location header to order the wells (i.e., 1st set of standards, 2nd set of standards, samples).

10.4.3.15.3.2 Select and copy all wells

10.4.3.15.4 Paste special into Quant Results section of the DNA Batch Workbook

10.4.3.15.4.1 Wrap text in cells containing “Sample Type” and “[Auto] ng/µL”

10.4.3.15.4.2 Change from scientific notation to general in the cells under "[Auto] ng/µL" and "[Y] ng/µL"

10.4.3.15.5 In the Plexor Software

10.4.3.15.5.1 File > Save Analysis File as New (.aan)

10.4.3.15.5.2 G Drive (G:) > Instrument- DNA > Plexor Projects > *Year* > Project

10.4.4 Limitations

10.4.4.1 See 10.5 Plexor HY Interpretation for additional information.

10.4.5 References

10.4.5.1 Applied Biosystems. 2006. Validation Using SDS Software Version 1.2.3 on the Applied Biosystems 7500 Real-Time PCR System and the ABI PRISM® 7000 Sequence Detection System: User Bulletin.

10.4.5.2 Krenke, Benjamin E., et al. Developmental Validation of a Real-Time PCR Assay for the Simultaneous Quantification of Total Human and Male DNA: Developmental Validation of the Plexor® HY System. Promega Corporation, 2007.

10.4.5.3 Plexor HY Technical Manual (May 2013). Plexor® HY System for the Applied Biosystems 7500 and 7500 FAST Real-Time PCR Systems. Promega, USA.

10.4.5.4 Internal Validation with Plexor® HY and 7500 Real-Time PCR System: (October 2014), Sorenson Forensics at MNPD-CL Forensic Biology Unit.

10.5 Plexor® HY Interpretation

10.5.1 Scope

10.5.1.1 To define some limitations of the Plexor HY system data and the interpretation of samples post-quantitation analysis.

10.5.2 Standards and Controls

10.5.2.1 If the quantitation fails resulting in samples being reprocessed, reagent blanks must also be reprocessed.

10.5.3 Procedure

10.5.3.1 Data from samples are compared to data from DNA standards of similar autosomal concentration values. The IPC amplification is designed to be the most inhibition-sensitive or least robust amplification in the triplex.

10.5.3.2 To determine quality of each quantitation value, look at the autosomal concentration in ng/µl of sample and find the IPC Cq for this sample. Then compare this IPC Cq to the value of the IPC Cq for the DNA standard that has the closest autosomal concentration in ng/µl.

10.5.3.2.1 If these values are similar, the sample quantitation is performing as expected with no indications of inhibition.

10.5.3.2.2 This may also be identified by looking at the Forensics report. If it indicates "Check IPC", this may indicate inhibition in a sample of possibly limited quantity. DNA concentration results for inhibited samples should be interpreted with caution.

10.5.3.2.2.1 Extremely high concentrations of human genomic DNA (>10 ng/µL) may result in a high IPC Cq and cause the "Check IPC" indicator to flag. It is unlikely that PCR inhibitors are present and the sample may be carried straight to amplification.

10.5.3.2.3 If the IPC for a sample does not cross the threshold and inhibition is suspected, dilutions will be created and re-quanted to correct the IPC. The dilution series for the re-quant set up is 1:2, 1:4, or both.

10.5.3.2.4 If the IPC for a reagent blank does not cross the threshold, the reagent blank must be re-quanted.

10.5.3.3 The amplification target must result in at least 10pg of DNA to proceed with autosomal amplification.

10.5.3.4 Samples being processed using Y-screening will be analyzed prior to proceeding to amplification. Depending on the case scenario and the probative information being collected from the sample, an extracted evidence sample having a Y quantitation value of 0ng/µl or N/A as displayed on the Forensic Report may not be further processed and may be stopped at quant.

10.5.3.5 It may be necessary to evaluate Auto/Y ratio to determine which amplification technology to use post-quantitation. Due to multi-copy target regions, there may be some variation observed in the Auto/Y ratio of a single male sample. See the below chart for various ranges of Auto/Y ratio (based on internal validation):

Comparable autosomal male profiles obtained	May produce comparable autosomal male profiles	Will not be forwarded for autosomal amplification
Less than or equal to 20/1	Greater than 20/1 less than or equal to 100/1	Greater than 100/1

10.5.3.6 The above chart may be used in deciding which samples to forward for amplification. For cases associated with a single assailant and no consensual sex partner within 5 days, one sample in the less than or equal to 20/1 range may be forwarded for autosomal amplification when available. If no samples exhibit a 20/1 ratio, all samples less than or equal to 100/1 should be forwarded for autosomal amplification. For cases associated with multiple assailants, a consensual sex partner within 5 days of the assault, or number of assailants is unknown; all samples exhibiting a less than 100/1 ratio should be forwarded for autosomal amplification. When the Auto/Y ratio for a sample is greater than 100/1, the sample will not be considered for autosomal STR amplification.

10.5.3.7 When a sample exhibits a Y melt that does not cross the threshold, processing of the sample will routinely stop at quantitation due to the limited quantitation data (see QMS ID 26012). The sample will be reported stating no determinations will be made regarding the presence of male DNA in the sample.

10.5.3.8 When all evidence in a case stops at the quantitation step, associated reference samples may also be stopped, provided that the samples are not eligible for the DNA database. The report will reflect that amplifiable DNA was obtained, but that no further testing was conducted.

10.5.4 Limitations

10.5.4.1 Plexor HY is limited in its ability to indicate the quality of the DNA. Low level inhibition may not be indicated on the quantitation. Plexor HY does not possess an indicator for degradation. The quantitative system allows for an estimate of the amount of DNA present in the extract. Validation data showed a 2-3 fold variance in the quantitation data from the same sample.

10.5.5 Safety

10.5.5.1 Caution should be used when handling chemicals and personal protective equipment should be used at all times.

10.5.6 References

10.5.6.1 Applied Biosystems. 2006. Validation Using SDS Software Version 1.2.3 on the Applied Biosystems 7500 Real-Time PCR System and the ABI PRISM® 7000 Sequence Detection System: User Bulletin.

10.5.6.2 Krenke, Benjamin E., et al. Developmental Validation of a Real-Time PCR Assay for the Simultaneous Quantification of Total Human and Male DNA: Developmental Validation of the Plexor® HY System. Promega Corporation, 2007.

10.5.6.3 Plexor HY Technical Manual (May 2013). Plexor® HY System for the Applied Biosystems 7500 and 7500 FAST Real-Time PCR Systems. Promeg, USA.

10.5.6.4 Internal Validation with Plexor® HY and 7500 Real-Time PCR System: (October 2014), Sorenson Forensics at MNPD-CL Forensic Biology Unit.

11. PowerPlex Fusion System

11.1 Extracted Samples

11.1.1 Scope

11.1.1.1 To describe the process by which casework samples that have been previously extracted are amplified at the following loci utilizing the PowerPlex Fusion System: D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, DYS391, D8S1179, D12S391, D19S433, FGA, D22S1045, and the gender marker Amelogenin.

11.1.2 Equipment/Materials/Reagents

11.1.2.1 PowerPlex Fusion System

11.1.2.1.1 PowerPlex® Fusion 5X Master Mix

11.1.2.1.2 PowerPlex® Fusion 5X Primer Pair Mix

11.1.2.1.3 2800M Control DNA, 10ng/µl

11.1.2.1.4 Water, Amplification Grade

11.1.2.2 1.5mL microcentrifuge tube

11.1.2.3 96-well reaction plate or 0.2mL amplification tubes

11.1.2.4 Vortex

11.1.2.5 Centrifuge

11.1.2.6 TE Buffer

11.1.2.7 8-Strip Caps

11.1.2.8 Veriti Thermal Cyclers

11.1.2.9 Pipettes

11.1.2.10 Pipette Tips

11.1.2.11 2800M Control DNA, 10ng/ul (purchased separately from PowerPlex Fusion System)

11.1.3 Standards and Controls

11.1.3.1 Positive and negative amplification controls must be co-amplified with each batch of samples. For the positive control, the 2800M Control DNA will be utilized. After its initial thaw, the 2800M Control DNA will be stored at 2-8°C. The diluted positive control should be made daily and stored at 2-8°C. The same lot of TE buffer that was used to dilute the samples will be used as the negative control.

11.1.3.2 At a minimum, one reagent blank must be co-amplified with each extraction batch. Reagent blanks must be amplified using the same testing kit, instrument model and concentration conditions as required by the sample(s) containing the least amount of DNA.

11.1.3.2.1 If multiple reagent blanks are present for each extraction batch, then amplify the reagent blank that demonstrates the highest quantified DNA concentration. If both reagent blanks demonstrate the same value, then either blank may be amplified.

11.1.3.3 When all samples in the batch stop at quantitation, the blank may be stopped at quant if all samples result in N/A. If any sample in the batch produce s a quantitation value (autosomal and/or Y), the blank must be carried through to amplification and analysis.

11.1.3.4 Refer to Section 13.2.3 of the MNPD-CL Forensic Biology Technical Procedures Manual for the criteria of passing controls.

11.1.4 Calibration

11.1.4.1 The Veriti thermal cyclers must be calibrated annually. Refer to the Maintenance and Calibration Protocols of the MNPD-CL Forensic Biology Quality Manual.

11.1.4.2 Each lot of Fusion kits will be quality control checked before being used in casework. Refer to Reagent Quality Control of the MNPD-CL Forensic Biology Quality Manual.

11.1.5 Procedure

11.1.5.1 Obtain and thaw the PowerPlex Fusion System at room temperature.

11.1.5.2 Normalization

11.1.5.2.1 Thaw, vortex, and pulse spin samples.

11.1.5.2.2 Proceed to Manual or Qiagility normalization procedure.

11.1.5.2.3 Manual

11.1.5.2.3.1 The 2800M positive control DNA and some samples will need to be diluted to achieve the appropriate input target value for the amplification reaction. TE buffer will be used to dilute the positive control and samples being amplified. Initial target values should not initially exceed 1.0ng. Upon reamplification a target value up to 2.0ng is allowed.

11.1.5.2.3.1.1 The following is an example of a normalization calculation. The example calculation assumes that only 1ul of quantified extract is used, the amplification target is 0.5ng, and 15ul of the diluted extract will be added to the amplification

reaction. The DNA Batch Workbook calculates normalizations in the manner described in this example. Note: calculated values are approximate and can be rounded to the nearest microliter.

11.1.5.2.3.1.1.1 To obtain the desired dilution concentration (i.e., target dilution) the desired amplification target (0.5ng) is divided by the desired amplification input volume (15ul).

11.1.5.2.3.1.1.1.1 Target dilution = 0.5ng/15ul = 0.0333ng/ul

11.1.5.2.3.1.1.2 To obtain the volume of TE needed to dilute the quantified extract, divide the extract's quantitation value (ng/µL) by the target dilution and then subtract the input volume of quantified extract.

11.1.5.2.3.1.1.2.1 Volume of TE = (*XYZ* ng/ul / 0.0333ng/ul) - 1ul. Ensure that the total volume is a minimum of 20µL.

11.1.5.2.3.1.1.3 In a dilution tube (should use a sterile tube that will house the necessary volumes) add 1ul of quantified extract and the calculated volume of TE. 15µL of this dilution will be added to the amplification reaction. If the total volume is less than 15µL, then multiply the volume of TE needed and the extract volume by the same factor until they add up to more than 15µL. For example, if the total volume is 5µL (1µL extract and 4µL TE buffer), multiply both values by 4 (4µL extract and 16µL TE buffer for a total of 20µL).

11.1.5.2.3.2 Proceed to the Amplification Set-up procedure.

11.1.5.2.4 QIAgility

11.1.5.2.4.1 Preparation Using the DNA Batch Workbook

11.1.5.2.4.1.1 Upon entering sample and quantification results information into the DNA Batch Workbook, the "Norm-QIA" tab will populate. Save the information in the "Norm-QIA" tab as a .txt file (tab delimited). (Instruction will be listed at the top of the excel sheet in this tab.)

11.1.5.2.5 QIAgility Instrument

11.1.5.2.5.1 **NOTE**: The Qiagility can only normalize samples ranging from 0.05 ng/µl and 10ng/µl. If a sample is greater than 10ng/µl, the sample can be diluted and placed on

the Qiagility with the calculated amount noted as the sample's DNA concentration on the.txt file.

11.1.5.2.5.2 Turn on computer.

11.1.5.2.5.3 Turn on instrument.

11.1.5.2.5.4 Launch QIAgility Software v4.15.1. The QIAgility instrument lid must be closed for the software and hardware to initialize upon start-up and for a run to proceed.

11.1.5.2.5.5 Open the Normalization protocol on the QIAgility by going to "File" at the top left of the software screen, click "Open," and find the "Normalization" protocol.

11.1.5.2.5.6 In the software, select the sample rack position C2. Click on the "Import" button and import the .txt file generated for your run. Then find the "Banks" section and select number "2" in the "Banks Specified in Column" option. Then click "Finished" button.

11.1.5.2.5.7 Load samples tubes into the 4 x 8 well sample racks in the Sample Block (C2).

11.1.5.2.5.8 Select the sample rack position (A2). Select the normalize samples Diluent Bank 2 module and click the edit button. Change the "First Well Number" to the beginning well number where the samples of Bank 2 begin on sample rack (C2). Then do this again for the remaining sample banks.

11.1.5.2.5.9 Load labeled empty 1.5mL flip cap tubes (in the same order as the sample tubes) into the 4 x 8 well sample racks in the sample rack position (A2).

11.1.5.2.5.10 Move the cursor over to Mix Plate (M1) position (A). A pop-up box will appear listing the amount of diluent that is needed for the run. Add this amount of diluent (TE Buffer) to a Qiagen 5ml diluent tube and place it on the Mix Plate (M1) at position (A).

11.1.5.2.5.11 Click on the green start arrow on the toolbar or select "Control/Start".

11.1.5.2.5.12 Check the pre-run report to verify the location and amount/volume of consumables and liquids that are required on the worktable for completion of the loaded run file.

11.1.5.2.5.13 The pre-run "Checklist" dialog box will appear. If no warnings or errors are listed, select the boxes to continue and click "OK" to start the run. If errors are listed, user intervention is required at this step.

11.1.5.2.5.14 Follow any prompted action pop-up windows during the duration of the normalization protocol.

11.1.5.2.5.15 **NOTE**: Care must be taken when opening the QIAgility lid during a run. It takes up to 10 seconds for the instrument to complete its current movement and for the pause to take effect.

11.1.5.2.5.16 Upon completion of the run, a "Post-run report" will appear. Save the "Post-run report" for the quant set up for the casefile.

11.1.5.2.5.17 Remove the now normalized samples on sample rack position (A2) and proceed to the Amplification Set-up procedure.

11.1.5.3 Amplification Set-up

11.1.5.3.1 Vortex the 5X Master Mix and 5X Primer Pair Mix for 5-15 seconds.

11.1.5.3.2 Prepare the amplification reaction mix in a clean 1.5mL or 2.0mL microcentrifuge tube. To prepare the reaction mix, count the number of samples to be amplified (including positive and negative controls). Add four to this total count for pipetting error. Multiply this value by five. The resulting value will be the volume (ul) of 5X Master Mix and 5X Primer Mix needed for the reaction mix. Add the calculated volumes of 5X Master Mix and 5X Primer Mix to the tube.

11.1.5.3.3 Vortex the amplification reaction mix.

11.1.5.3.4 Proceed to the Manual or QIAgility amplification procedure.

11.1.5.3.4.1Manual

11.1.5.3.4.1.1 Dispense 10µL of the reaction mix into the appropriate wells of a 96-well reaction plate, skipping wells assigned for Ladders. 0.2mL amplification tubes may also be used.

11.1.5.3.4.1.2 Following the addition of reaction mix and prior to the addition of sample, check to ensure that reaction mix has been added to all appropriate wells.

11.1.5.3.4.1.3 Add 15 µL of DNA extract or normalized DNA extract dilution to the appropriate wells/tubes.

11.1.5.3.4.1.4 Add 15µL of TE (negative control) or diluted positive control to the appropriate well/tube.

11.1.5.3.4.1.5 Cap each column of the plate with strip caps as each column is completed. If using tubes, only one tube should be open at a time.

11.1.5.3.4.1.6 Proceed to Preparation of Plate for PCR.

11.1.5.3.4.2 QIAgility

11.1.5.3.4.2.1 Preparation Using the DNA Batch Workbook

11.1.5.3.4.2.1.1 Upon entering sample information into the DNA Batch Workbook, open the "Amp-QIA" tab. Save the information in the "Amp-QIA" tab as a .txt file (tab delimited). (Instruction will be listed at the top of the excel sheet in this tab.)

11.1.5.3.4.2.2 QIAgility Instrument

11.1.5.3.4.2.2.1 Turn on computer.

11.1.5.3.4.2.2.2 Turn on instrument.

11.1.5.3.4.2.2.3 Launch QIAgility Software v4.15.1. The QIAgility instrument lid must be closed for the software and hardware to initialize upon start-up and for a run to proceed.

11.1.5.3.4.2.2.4 Place prepared Powerplex Fusion Reaction Mix into position F on the Reagent Block (R1) of the QIAgility. Place a 1.5ml flip cap tube with water in the "Ladder Blank" position H on Reagent Block (R1).

11.1.5.3.4.2.2.5 Load samples tubes into the 4 x 8 well sample racks in the Sample Block (A2)

11.1.5.3.4.2.2.6 Place a 96 well optical plate in Reaction Block (C1).

11.1.5.3.4.2.2.7 Open the Amplification protocol by going to "File" at the top left of the software screen, click "Open," and find the "Amp (15)" protocol.

11.1.5.3.4.2.2.8 In the software, select the sample rack position A2. Click on the "Import" button and import the .txt file generated for your run.

11.1.5.3.4.2.2.9 Click on the green start arrow on the toolbar or select "Control/Start".

11.1.5.3.4.2.2.10 Check the pre-run report to verify the location and amount/volume of consumables and liquids that are required on the worktable for completion of the loaded run file.

11.1.5.3.4.2.2.11 The pre-run "Checklist" dialog box will appear. If no warnings or errors are listed, select the boxes to continue and click "OK" to start the run. If errors are listed, user intervention is required at this step.

11.1.5.3.4.2.2.12 Follow any prompted action pop-up windows during the duration of the protocol.

11.1.5.3.4.2.2.13 **NOTE**: Care must be taken when opening the QIAgility lid during a run. It takes up to 10 seconds for the instrument to complete its current movement and for the pause to take effect.

11.1.5.3.4.2.2.14 Upon completion of the run, a "Post-run report" will appear. Save the "Post-run report" for the amplification set up for the case file.

11.1.5.3.4.2.2.15 Proceed to the Preparation of Plate for PCR.

11.1.5.4 Preparation of Plate for PCR

11.1.5.4.1 Cap each column in the plate with sample in it with the 8-strip cap or cap each tube.

11.1.5.4.2 Briefly centrifuge the plate.

11.1.5.5 PCR

11.1.5.5.1 Log on to the thermal cycler using the following credentials:

11.1.5.5.1.1 Username: scientist

11.1.5.5.1.2 Password: scientist1

11.1.5.5.2 Load the samples onto the Veriti thermal cycler and close the lid. Select the "Powerplex Fusion 5C" method. Verify the program is set to 9700 Max mode and is as follows:

11.1.5.5.2.1 96°C for 1 min

11.1.5.5.2.2 Then, 30 cycles of:

11.1.5.5.2.2.1 94°C for 10 sec

11.1.5.5.2.2.2 59°C for 1 min

11.1.5.5.2.2.3 72°C for 30 sec

11.1.5.5.2.3 Then,

11.1.5.5.2.3.1 60°C for 10 min

11.1.5.5.2.3.2 4°C soak

11.1.5.5.3 Start the run.

11.1.5.5.4 Once the program is complete, the samples are ready for capillary electrophoresis or can be stored at -10°C to -25°C for future use.

11.1.5.5.5 The sequence of this procedure is not absolute. An analyst may choose to prepare the sample dilutions prior to preparing the amplification reaction mix or vice versa. An analyst may also choose to add TE buffer to the amplification tube or plate prior to dispensing the reaction mix.

11.1.6 Limitations

11.1.6.1 If necessary, the amplification input volume of DNA can be adjusted. If this is necessary, TE must be used to so that the total amplification input volume of DNA and TE

is 15ul. Prior approval must be obtained by the DNA Technical Leader in order to adjust the input volume.

11.1.6.2 This procedure is not optimized for direct amplification of known standards that have not been previously extracted.

11.1.7 Safety

11.1.7.1 Caution should be used when handling chemicals and personal protective equipment should be used at all times.

11.1.7.2 The robotic arm of the QIAgility instrument moves during position calibration while the instrument lid is raised. Never click any buttons while parts of your body are within the instrument workspace.

11.1.8 References

11.1.8.1 Developmental Validation of the PowerPlex® Fusion System for Analysis of Casework and Reference Samples: A 24-locus Multiplex for New Database Standards (2014). Forensic Science International: Genetics, web.

11.1.8.2 Internal Validation of Promega PowerPlex® Fusion using a Veriti

11.1.8.3 Thermal Cycler and 3500 Series Genetic Analyzer (August 2014), Sorenson Forensics at MNPD-CL Forensic Biology Unit.

11.1.8.4 PowerPlex Fusion System Technical Manual (2012). Instructions for use of Products DC2402 and DC2408. Promega, Madison, WI.

11.1.8.5 Internal Validation with QIAgility: (2015), Sorenson Forensics at MNPD-CL Forensic Biology Unit.

11.1.8.6 QIAgility® User's Manual (June 2013). QIAGEN.

11.2 Direct Amplification of Swabs

11.2.1 Scope

11.2.1.1 To describe the process by which swab samples undergo direct amplification at the following loci utilizing the PowerPlex Fusion System: D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, DYS391, D8S1179, D12S391, D19S433, FGA, D22S1045, and the gender marker Amelogenin.

11.2.2 Equipment/Materials/Reagents

11.2.2.1 PowerPlex® Fusion System

11.2.2.2 SwabSolution™ Reagent

11.2.2.3 1.5mL microcentrifuge tubes

11.2.2.4 96-well reaction plate or 0.2mL amplification tubes

11.2.2.5 Vortex

11.2.2.6 Centrifuge

11.2.2.7 Amplification grade water

11.2.2.8 Heat block

11.2.2.9 8-Strip Caps

11.2.2.10 Veriti Thermal Cyclers

11.2.3 Standards and Controls

11.2.3.1 Positive and negative amplification controls must be co-amplified with each batch of samples. For the positive control, the 2800M Control DNA that is supplied with the Fusion System will be utilized. The same lot of amplification grade water used in the master mix will be used as the negative control.

11.2.3.2 At a minimum, one reagent blank must be co-amplified with each extraction batch.

11.2.3.3 Refer to Section 13.2.3 of the MNPD-CL Forensic Biology Technical Procedures Manual for the criteria of passing controls.

11.2.4 Performance Checks

11.2.4.1 The Veriti thermal cyclers are performance checked annually. Refer to the Maintenance and Calibration Protocols of the MNPD-CL Forensic Biology Quality Manual.

11.2.4.2 Each lot of Fusion kits and SwabSolution™ Reagent will be performance checked before being used in casework. Refer to Reagent Quality Control of the MNPD-CL Forensic Biology Quality Manual.

11.2.5 Procedure

11.2.5.1 Set heat block to 70ºC and allow it to heat to temperature.

11.2.5.2 Place each buccal swab head in a separate 1.5mL tube. Add 1mL of SwabSolution™ Reagent to each buccal swab head. Place tubes in heat block, and incubate samples at 70ºC for 30 minutes.

11.2.5.2.1 **Note**: There is no need to vortex the tubes after addition of the SwabSolution™ Reagent, prior to or after the incubation.

11.2.5.3 Obtain and thaw the PowerPlex Fusion System at room temperature.

11.2.5.4 Vortex the 5X Master Mix and 5X Primer Pair Mix for 15 seconds.

11.2.5.5 Prepare the amplification master mix in a clean 1.5mL microcentrifuge tube. Count the number of samples to be amplified (including positive and negative control) and add four for pipetting error. For this number, add 13µL amplification grade water, 5µL of the 5X Master Mix and 5µL of the 5X Primer Mix to the tube.

11.2.5.6 Vortex the amplification master mix. Dispense 23µL of the master mix into the appropriate well of a 96-well reaction plate or the appropriate labeled 0.2mL amplification tube for each sample.

11.2.5.7 Dispense 2µL of swab extract for each sample into the appropriate well or tube containing 23µL of master mix.

11.2.5.8 For the positive control, vortex the tube of 2800M DNA, then dilute to the appropriate target (total target of 1-2 ng) using amplification grade water.

11.2.5.9 For the negative control, add 2µL of amplification grade water to the appropriate well or tube containing 23µL of master mix.

11.2.5.10 Cap each column in the plate with sample in it with the 8-strip cap or cap each tube.

11.2.5.11 Briefly centrifuge the plate.

11.2.5.12 Load the samples onto the Veriti thermal cycler, close the lid, and start the Fusion program for direct amplification samples. Verify the program is set to 9700 Max mode and is as follows:

11.2.5.12.1 96°C for 1 min

11.2.5.12.2 Then, 27 cycles of:

11.2.5.12.2.1 94°C for 10 sec 59°C for 1 min

11.2.5.12.2.2 72°C for 30 sec

11.2.5.12.3 Then,

11.2.5.12.3.1 60°C for 20 min

11.2.5.12.3.2 4°C soak

11.2.5.13 Once the program is complete, the samples are ready for capillary electrophoresis or can be stored at -10°C to -25°C for future use.

11.2.6 Calculations

11.2.6.1 The 2800M positive control DNA will need to be diluted to achieve the appropriate input target value for the amplification reaction. Use these calculations for determining the dilution factor of the 2800M to be amplified:

11.2.6.1.1 For a 2ng target dilution, quant value (ng/uL)/1 = total volume in uL.

11.2.6.1.2 Total volume – 1uL undiluted 2800M = volume of amplification grade water needed.

11.2.7 Limitations

11.2.7.1 This procedure is not optimized for amplification of extracted samples. Refer to Section 11.1.5 for that procedure.

11.2.8 Safety

11.2.8.1 Caution should be used when handling chemicals and personal protective equipment should be used at all times.

11.2.9 References

11.2.9.1 Developmental Validation of the PowerPlex® Fusion System for Analysis of Casework and Reference Samples: A 24-locus Multiplex for New Database Standards (2014). Forensic Science International: Genetics, web.

11.2.9.2 Internal Validation of Direct Amplification of Swabs using the Promega PowerPlex® Fusion System (2014), Sorenson Forensics at MNPD-CL Forensic Biology Unit.

11.2.9.3 PowerPlex® Fusion System Technical Manual (2012). Instructions for use of Products DC2402 and DC2408. Promega, Madison, WI.

11.2.9.4 SwabSolution™ Kit Technical Manual (2013). Instructions for use of Product DC8271. Promega, Madison, WI.

12. Capillary Electrophoresis Protocols

12.1 Scope

12.1.1 To describe the process of separating DNA fragments via capillary electrophoresis utilizing the Applied Biosystems 3500 Genetic Analyzer.

12.2 Equipment/Materials/Reagents

12.2.1 Applied Biosystems 3500 series Genetic Analyzer

12.2.2 Applied Biosystems 3500 series Genetic Analyzer Data Collection Software version 4.0.1

12.2.3 Vortex

12.2.4 Centrifuge

12.2.5 Microcentrifuge tubes

12.2.6 Pipettes

12.2.7 Pipette tips

12.2.8 Hi-Di formamide

12.2.9 WEN Internal Lane Standard 500 (ILS)

12.2.10 POP-4 polymer

12.2.11 Anode buffer container

12.2.12 Cathode buffer container

12.2.13 3500 series 96-well plate base/retainer set

12.2.14 96-well plate

12.2.15 96-well plate septa

12.2.16 PowerPlex Fusion and/or PowerPlex Y23 Allelic Ladder

12.3 Standards and Controls

12.3.1 The WEN Internal Lane Standard 500 (ILS) will be co-injected with every sample/allelic ladder. The ILS contains DNA fragments of known sizes that are used to size allelic ladder(s) which are then compared to the PCR products of the samples that have also been sized in base pairs with their own ILS to allow allele designation.

12.3.2 At a minimum, one allelic ladder must be injected for each 96-well plate. It is recommended that an allelic ladder be run every other injection to compensate for

potential migration differences. The allelic ladder is used to assign allele calls to the samples.

12.4 Calibration

12.4.1 The Applied Biosystems 3500 series Genetic Analyzer must undergo annual maintenance. Refer to the Maintenance and Calibration Protocols of the MNPD-CL Forensic Biology Quality Manual for the maintenance and calibration procedures. When the annual maintenance is complete, the instrument will be performance checked prior to running casework samples.

12.4.2 All reagent components used in capillary electrophoresis will be quality control checked prior to being used in casework. Refer to Reagent Quality Control of the MNPD-CL Forensic Biology Quality Manual.

12.5 Procedure

12.5.1 Turn on the heat block and pre-heat to 95°C.

12.5.2 If not previously done, thaw the ILS and allelic ladder(s) to room temperature. Also, thaw an aliquot of Hi-Di formamide to room temperature.

12.5.3 Vortex the ILS, ladders, and formamide for 5-15 seconds.

12.5.4 To prepare a master mix of the ILS and formamide, count the number of samples to be injected (including allelic ladders, reagent blanks, and positive and negative amplification controls) and add four for pipetting error.

12.5.4.1 To obtain the necessary volume of the ILS, multiply the total count by the volume of ILS (0.5 – 1.0 µL of ILS per sample).

12.5.4.2 To obtain the necessary volume of formamide, multiply the total count by 10 (10 µL of formamide per sample).

12.5.4.3 Add the calculated volumes of formamide and ILS in a 1.5mL microcentrifuge tube.

12.5.5 Vortex the master mix for 5-15 seconds.

12.5.6 Proceed to the Manual Plating or QIAgility Plating CE procedure.

12.5.7 Manual Plating

12.5.7.1 Dispense 10µL of the master mix into the appropriate wells of a labeled 96-well plate. If a column on the plate has at least one sample being injected, then dispense master mix

into all eight wells. Wells that do not contain a sample can be filled with 10ul of formamide.

12.5.7.2 Add 1µL of the previously amplified samples or allelic ladder to the appropriate wells of the 96-well plate.

12.5.7.3 **NOTE**: Amplified product may be diluted in ultrapure water when data generated exhibits oversaturation and/or pull-up. 1µL of the dilution should be added to the 96-well plate prepared for CE.

12.5.7.4 Proceed to Prepare the plate for placement on the CE instrument.

12.5.8 QIAgility Plating

12.5.8.1 Preparation Using the DNA Batch Workbook

12.5.8.1.1 In the DNA Batch Workbook open the "CE- QIA" tab. Save the information in the Amp Set-Up 1 tab or Amp Set-Up 2 tab as a .txt file (tab delimited). (Instruction will be listed at the top of the excel sheet in this tab.)

12.5.8.1.2 **Note**: To combine amplification plates on a CE plate, separate .txt files must be saved for each, with one named Plate1 (Ex. "Fusion_Plate1_Bank1") and the other named Plate2 (Ex. "Fusion_Plate2_Bank1.")

12.5.8.1.2.1 The "Plate1" or "Plate 2" designation denotes which of the two plate sample areas on the QIAgility work deck is being utilized.

12.5.8.2 QIAgility Instructions

12.5.8.2.1 Turn on computer.

12.5.8.2.2 Turn on instrument.

12.5.8.2.3 Launch QIAgility Software v4.15.1. The QIAgility instrument lid must be closed for the software and hardware to initialize upon start-up and for a run to proceed.

12.5.8.2.4 Place prepared formamide and ILS Mix into position N on the Reagent Block (R1) of the QIAgility. Place the Powerplex Fusion Ladder into position P on Reagent Block (R1). If Powerplex Y23 samples are present, place the Poweplex Y23 ladder into position O.

12.5.8.2.5 Load an amplification plate or samples into the Sample Block (B1) or (C1).

12.5.8.2.6 Place a 96 well optical plate in Reaction Block (B2).

12.5.8.2.7 Open the CE setup protocol by going to "File" at the top left of the software screen, click "Open," and find the "Post Setup-consolidated" protocol.

12.5.8.2.8 In the software, select the sample rack position (B1) or (C1). Click on the "Import" button and import the .txt file generated for your run.

12.5.8.2.8.1 **Note**: This is where the user will designate "Plate1" and "Plate2." Therefore, if the user designates a set of samples for "Plate1" and imports those to sample rack position (B1), then samples for "Plate2" must be imported to sample rack position (C1).

12.5.8.2.9 Click on the green start arrow on the toolbar or select "Control/Start".

12.5.8.2.10 Check the pre-run report to verify the location and amount/volume of consumables and liquids that are required on the worktable for completion of the loaded run file.

12.5.8.2.11 The pre-run "Checklist" dialog box will appear. If no warnings or errors are listed, select the boxes to continue and click "OK" to start the run. If errors are listed, user intervention is required at this step.

12.5.8.2.12 Follow any prompted action pop-up windows during the duration of the protocol.

12.5.8.2.13 Care must be taken when opening the QIAgility lid during a run. It takes up to 10 seconds for the instrument to complete its current movement and for the pause to take effect.

12.5.8.2.14 Upon completion of the run, a "Post-run report" will appear. Save the "Post-run report" for the casefile.

12.5.8.2.15 Remove the 96 well optical plate in Reaction Block (B2) and proceed to Prepare the plate for placement on the CE instrument.

12.5.9 Prepare the plate for placement on the CE instrument

12.5.9.1 Place a 96-well plate septa onto the plate, ensuring that the wells are aligned with the holes in the septa.

12.5.9.2 Centrifuge the plate briefly to force the contents to the bottom of the wells and to remove bubbles.

12.5.9.3 Denature samples at 95°C for 3 minutes, and then immediately chill on crushed ice or a freezer plate block or in an ice-water bath for 3 minutes. Denature samples just prior to loading the instrument.

12.5.10 CE

12.5.10.1 Perform a shut-down and restart on the first use of the day.

12.5.10.2 Turn off the computer.

12.5.10.3 Turn off the instrument.

12.5.10.4 After one minute, turn on the computer, but do not log in.

12.5.10.5 Turn on the instrument and wait for the green light to stop flashing.

12.5.10.6 Log into the computer (Username: Administrator / Password: Administrator).

12.5.10.7 Wait for all connections to be made indicated by a green check mark in the task bar.

12.5.10.8 Launch the Applied Biosystems 3500 series Genetic Analyzer Data Collection Software v4.0.1. Login to the software using the following credentials:

12.5.10.8.1 Username: Scientist

12.5.10.8.2 Password: Scientist1

12.5.10.9 Ensure that the polymer, cathode buffer container, and anode buffer container contain enough reagents to complete all injections and are within the validated expiration period. Also, verify that the necessary maintenance procedures have been completed according to the Maintenance and Calibration Protocols of the MNPD-CL Forensic Biology Quality Manual.

Reagent	Allowance on 3500		
Polymer	≤ 14 days	≤ 384 samples	Expiration Date
Anode Buffer	≤ 14 days	≤ 240 injections	Expiration Date
Cathode Buffer	≤ 14 days	≤ 240 injections	Expiration Date
Capillary Array	≤ 120 injections		

12.5.10.10 Place the plate into a 3500 series plate retainer/base set. Load the plate onto the instrument with the notched corner of the plate aligned with the notched corner of the autosampler tray.

12.5.10.11 From the Dashboard click the “Create New Plate” button. Enter the plate name. Select “96” for Number of Wells, “HID” for Plate Type, “36” cm for Capillary Length, and “POP4” for Polymer. At the bottom of the page, click Assign Plate Contents.

12.5.10.12 Proceed to Manual Sample Entry or DNA Batch Workbook Import.

12.5.10.13 Manual Sample Entry

12.5.10.13.1 Type each sample or control name in the appropriate well. Click on Add from Library under Assays and select “PowerPlex Fusion WENILS 5C Casework Long”.

12.5.10.13.2 Click on Add from Library under File Name Convention and select “MNPD”.

12.5.10.13.3 Click on Add from Library under Results Group and select “MNPD Fusion”.

12.5.10.13.4 To assign the assay, file name convention, and results group to the plate, select all wells being used and check the boxes beside each option just imported from the libraries.

12.5.10.13.5 Click the Table View tab.

12.5.10.13.6 For each control, select the appropriate control designation in the dropdown menu under sample type.

12.5.10.13.7 Proceed to section 12.5.8.15 Begin the run.

12.5.10.14 DNA Batch Workbook Import

12.5.10.14.1 In the data collection software, import the plate's Text Tab Delimited file from the DNA Batch Workbook (Created in the "3500-template" tab. Instructions will be listed at the top of the excel sheet in this tab. Save file to a USB drive, and transfer USB to instrument computer.) Under "Assign Plate Contents", select "Import"

12.5.10.14.2 Navigate to the .txt file in the USB drive and select it. Enter or verify the plate name. Ensure the plate contents have populated correctly from the import file and that the Assay "PowerPlex Fusion WENILS 5C Casework Long", File Name Convention "MNPD", and Results Group "MNPD Fusion" have been selected for each sample well in the run. If desired, update the sample type for controls (i.e., allelic ladder, positive control). Sample types can be edited in GeneMapper as well.

12.5.10.14.3 Proceed to section 12.5.8.15 Begin the run.

12.5.10.15 Begin the run

12.5.10.15.1 Click "Link for Run" button at the bottom of the page and then click "OK". In the "Load Plates for Run" screen, ensure that the Run File Name of the plate is associated with the correct side of the autosampler tray.

12.5.10.15.2 Click "Create Injection List" and a "Preview Run Screen" will appear with a list of injections and the plate layout on the right hand side. Review the injection list and sample order (may be altered if necessary).

12.5.10.15.3 Click "Start Run" at the bottom of the screen.

12.5.10.15.4 When the run is finished, go to the Data shortcut on the desktop of the 3500 computer. Copy the Run file with your data to a USB drive. Take the USB to the other networked computer and copy the data to the G drive (G:\Instrument – DNA\Run Files\3500 Genetic Analyzer\Joker). Save under the specific year and month associated with the run.

12.6 Limitations

12.6.1 Repeated freeze/thaw cycles should be avoided with formamide as this may cause the product to break down into formic acid and formate. Formate ions migrate preferentially into the capillary during electrokinetic injection causing loss of signal intensity.

12.7 Safety

12.7.1 Formamide is a known teratogen. Work should be conducted inside a safety enclosure when handling this reagent. Personal protective equipment should be worn at all times. Formamide should be disposed of in the biohazard waste.

12.7.2 The robotic arm of the QIAgility instrument moves during position calibration while the instrument lid is raised. Never click any buttons while parts of your body are within the instrument workspace.

12.8 References

12.8.1 Applied Biosystems 3500/3500xL Genetic Analyzer User Guide (2010). Applied Biosystems, Foster City, CA.

12.8.2 Applied Biosystems. 2011. Applied Biosystems 3500/3500xL Genetic Analyzer: User Bulletin.

12.8.3 Internal Validation of Promega PowerPlex® Fusion using a Veriti Thermal Cycler and 3500 Series Genetic Analyzer (August 2014), Sorenson Forensics at MNPD-CL Forensic Biology Unit.

12.8.4 PowerPlex Fusion System Technical Manual (2012). Instructions for use of Products DC2402 and DC2408. Promega, Madison, WI.

12.8.5 Internal Validation with QIAgility: : (2015), Sorenson Forensics at MNPD-CL Forensic Biology Unit.

12.8.6 QIAgility® User's Manual (June 2013). QIAGEN.

13. Data Analysis

13.1 GeneMapper® ID-X Software Setup and Functionality

13.1.1 Scope

13.1.1.1 To describe the importing of raw data obtained from the Applied Biosystems 3500 Genetic Analyzer using the appropriate version of the GeneMapper® ID-X Software.

13.1.2 Equipment/Materials/Reagents

13.1.2.1 GeneMapper® ID-X Software v1.6

13.1.3 Standards and Controls

13.1.3.1 Each folder in a project must contain at least one allelic ladder for proper genotyping.

13.1.4 Procedure

13.1.4.1 Double-click  (GeneMapper® ID-X) on the desktop to launch the software.

13.1.4.2 In the Login to GeneMapper® ID-X dialog box, enter User Name and Password, then click OK.

13.1.4.3 Select File, then New Project.

13.1.4.4 In the new Project window, click  (Add Samples to Project).

13.1.4.5 Browse to the location of the run files (This PC > Crime Lab Backup (G:) > Run Files > 3500 Genetic Analyzer > Joker > Year > Month). Highlight desired files/CE folder, then select “Add To List” followed by “Add”.

13.1.4.6 If not done during CE setup, in the Sample Type column, use the drop-down menu to select Allelic Ladder, Sample, Positive Control, or Negative Control as appropriate for each sample.

13.1.4.6.1 Every folder in the project must contain at least one allelic ladder for proper genotyping.

13.1.4.7 In the Analysis Method column, select “Powerplex Fusion MNPD 2017”.

13.1.4.7.1 Analytical Thresholds:

13.1.4.7.1.1 Blue: 200 RFU

13.1.4.7.1.2 Green: 200 RFU

13.1.4.7.1.3 Yellow: 250 RFU

13.1.4.7.1.4 Red: 250 RFU

13.1.4.7.1.5 Orange: 100 RFU

13.1.4.8 In the Panel column, select “PowerPlex_Fusion_Panels_IDX_v2.0”.

13.1.4.9 Ensure that “WEN_ILS_500_IDX” is selected in the Size Standard column.

13.1.4.10 Highlight “Analysis Method”, “Panel”, and “Size Standard” columns and press “Ctrl + D” to apply selections to all samples.

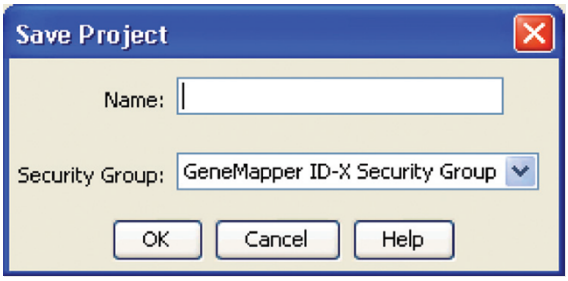
13.1.4.11 In the Custom Control column, select “2800 M” for each positive control.

13.1.4.12 Click  (Analyze) to start data analysis.

13.1.4.13 A pop-up window titled “Samples Not Normalized” opens. Click “OK”.

13.1.4.14 If all analysis requirements are met, the “Save Project” dialog box will open.

13.1.4.15 In the “Save Project” dialog box, enter the project name. This should have the same name as the CE plate and CE run. Choose the applicable security group, then select OK.

13.1.4.15.1 

13.1.4.15.1.1 If a “Disk Space Alert” window pops up, select “OK”.

13.1.4.15.2 Analysis will now begin.

13.1.4.15.3 The software identifies any conditions that may prevent analysis or cause unexpected results. The Analysis Summary (ARS) tab will open if at least one sample in the project does not meet one or more analysis requirements.

13.1.4.15.3.1 From the ARS, you may view the samples that do not meet the analysis requirements or continue with analysis by clicking the “Samples” tab.

13.1.4.15.4 Use the table setting “MNPD – Casework Table” when reviewing results.

13.1.4.16 Saving the Project:

13.1.4.16.1 When editing of samples has been completed (refer to Data Analysis and Editing section), click  (Save Project).

13.1.5 Limitations

13.1.5.1 Although a global forward stutter percentage is set for the Powerplex Fusion system in the analysis method, the analyst should note that the forward stutter filter is only applied to tetra repeats.

13.1.5.2 For PowerPlex Fusion data: Sizing of Penta E and DYS391 alleles ≥475 bases will not use Local Southern Method. For Penta E, alleles >24 will be labeled as "OL".

13.1.6 References

13.1.6.1 GeneMapper® ID-X Software Version 1.0 Getting Started Guide (October 2007).Applied Biosystems, Foster City, CA.

13.1.6.2 PowerPlex Fusion System Technical Manual (October 2012). Promega, Madison, WI.

13.1.6.3 PowerPlex Y23 System Technical Manual (July 2012). Promega, Madison, WI.

13.2 Data Analysis and Editing

13.2.1 Scope

13.2.1.1 To describe the process of analyzing the raw data obtained from the Applied Biosystems 3500 Genetic Analyzer using the appropriate version of the GeneMapper® ID-X Software .

13.2.2 Equipment/Materials/Reagents

13.2.2.1 GeneMapper® ID-X Software v1.6

13.2.2.2 HID data files from AB 3500

13.2.3 Procedure

13.2.3.1 To begin data analysis, samples must be added to the project to be analyzed.

13.2.3.1.1 Refer to GeneMapper® ID-X Software Setup and Functionality for how to analyze data.

13.2.3.2 Examine the internal size standard (ILS) for all samples and controls included in the project.

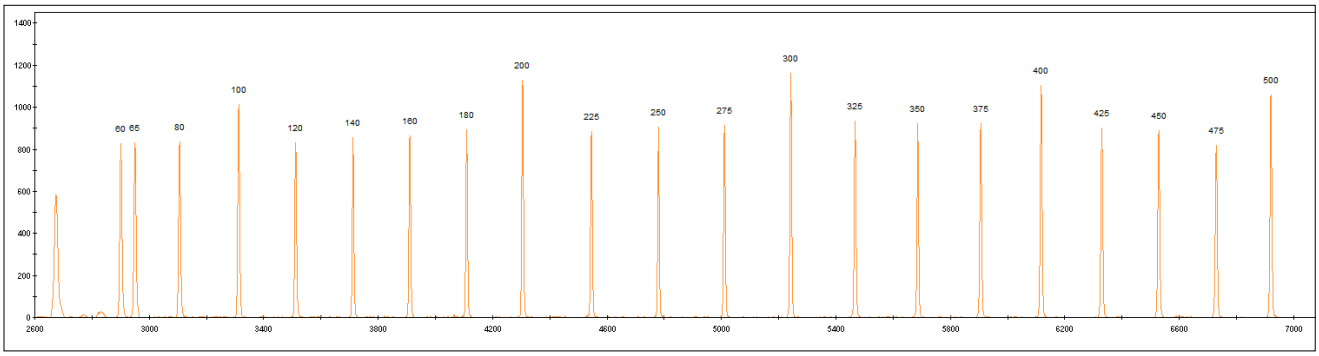
13.2.3.2.1 During peak detection and size-calling, the GeneMapper® ID-X Software matches an observed fragment peak from the size standard run with the sample to a corresponding size in the definition file.

13.2.3.2.2 Click on the Project node for the entire project being analyzed or the individual sample to be examined, then click (Size Match Editor) to view the peak assignments for the size standard peaks in the sample(s).

13.2.3.2.3 Alternatively, highlight the sample(s) then click (Display Plots). In the Samples plot, select the Check LIZ Standard Plot Setting from the drop-down list.

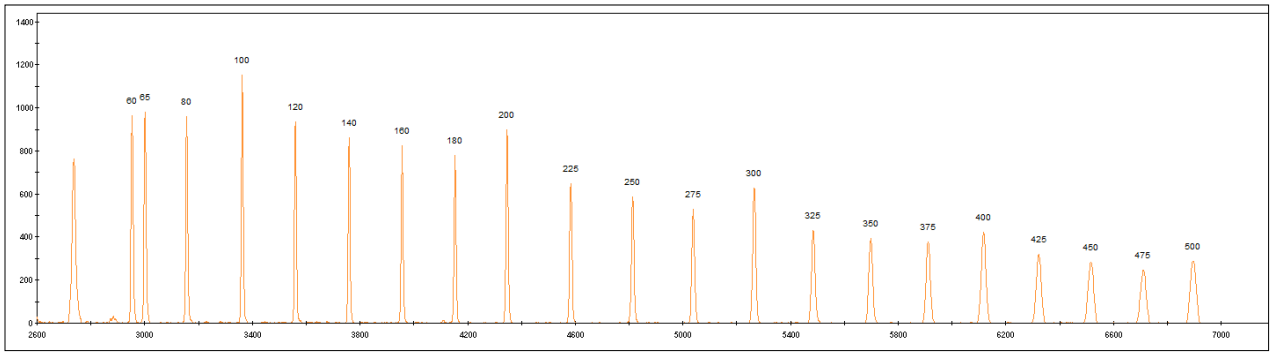
13.2.3.2.4 Check to determine that all peaks for the size standard are detected and labeled properly.

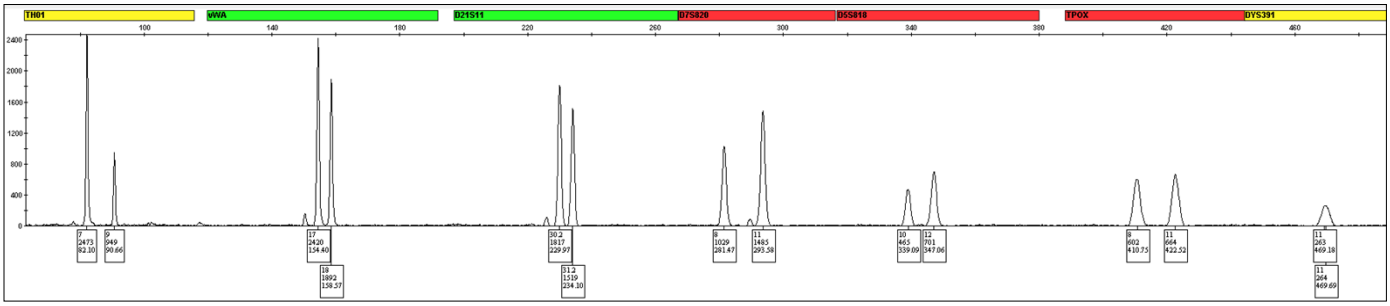
13.2.3.2.4.1 The sizes for the ILS fragments should be labeled as follows: 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, and 500 bases.



13.2.3.2.4.2 If peaks are incorrectly labeled, they may be edited by highlighting and right clicking on the peak to be edited in the Size Match Editor window. Select “Add”, “Delete”, or “Change Peak” as appropriate. Select “Apply” to accept changes. The project will need to be reanalyzed.

13.2.3.2.4.3 If a sample’s size standard is sloping to the right (decreasing in peak height and broadening of peaks) the resolution of the sample may be affected. This sample should be carefully reviewed as it may need to be reinjected or replated to obtain quality results. Poor resolution in a sample can appear as broad/wide allele peaks on the right side of the electropherogram.





13.2.3.3 Review the Allelic Ladder quality.

13.2.3.3.1 To accurately genotype samples, the GeneMapper® ID-X Software requires at least one allelic ladder sample per run folder. For a ladder to be used for data analysis, the sizing quality (SQ) and composite genotype quality (CGQ) flags must not be flagged (indicated as green squares).

13.2.3.3.1.1 **NOTE**: If a run folder contains multiple allelic ladder samples, the GeneMapper® ID-X Software calculates bin offsets using an average of all ladders that use the same panel within a run folder. Only Allelic Ladders processed with the associated samples will be used in the run project.

13.2.3.3.2 If the ladder does not pass, then click (Display Plots). When troubleshooting failed ladders, select “Allelic Ladder Analysis” from Plot Settings drop-down list.

13.2.3.3.3 Verify the ladder has the correctly called alleles for each locus and the peak morphology is characteristic of peaks (i.e., no broadening of peaks).

13.2.3.3.4 If a ladder does not call correctly, change the failed ladder(s) to ‘Sample’ and re-analyze the project. Only one ladder is required per project.

13.2.3.3.5 Since the software applies an average of the ladders to the samples, it may be necessary to remove outlying ladders by changing them to ‘Sample’ and re-analyzing the project. Only one ladder is required per project.

13.2.3.3.6 **Note**: If only one ladder is selected, this may lead to off-ladder allele calls in the samples.

13.2.3.4 Review of Positive Controls

13.2.3.4.1 During analysis of the project, the positive control sample should be selected as a custom control matching to 2800M. If the SQ and CGQ Quality Flags indicate passing (green squares), then the positive control has passed and may be used for analysis. If either of these flags does not pass, follow the steps below.

13.2.3.4.1.1 Select the Positive Control sample(s) in the Samples table, then click (Display Plots).

13.2.3.4.1.2 Select "Data Interpretation" from the Plot Setting drop-down list.

13.2.3.4.1.3 Verify that the DNA allelic designations gave the expected results.

STR Locus	2800M
Amelogenin	X, Y
D3S1358	17, 18
D1S1656	12, 13
D2S441	10, 14
D10S1248	13, 15
D13S317	9, 11
Penta E	7, 14
D16S539	9, 13
D18S51	16, 18
D2S1338	22, 25
CSF1PO	12, 12
Penta D	12, 13
TH01	6, 9.3
vWA	16, 19
D21S11	29, 31.2
D7S820	8, 11
D5S818	12, 12
TPOX	11, 11
DYS391	10
D8S1179	14, 15
D12S391	18, 23
D19S433	13, 14
FGA	20, 23
D22S1045	16, 16

Y-STR Locus	2800M
DYS576	18
DYS389I	14
DYS448	19
DYS389II	31
DYS19	14
DYS391	10
DYS481	22
DYS549	13
DYS533	12
DYS438	9
DYS437	14
DYS570	17
DYS635	21
DYS390	24
DYS439	12
DYS392	13
DYS643	10
DYS393	13
DYS458	17
DYS385a/b	13, 16
DYS456	17
Y-GATA-H4	11

13.2.3.4.1.4 A positive control is considered to pass when a full profile is obtained, with no more than 6 locations exhibiting peak height ratios below 60%. If dropout is detected in

the positive control, data may be used with the approval and guidance of the DNA Technical Leader.

13.2.3.4.1.5 If the positive control injected poorly, it can be re-injected or if necessary, the positive control sample can be re-prepared and injected. The original sample injections can be analyzed if upon re-inject/rerun the positive control gives the expected results.

13.2.3.4.1.6 Positive controls that do not meet passing criteria, remain in the project as a "Positive Control". Note failed positive controls in the associated comment box in GeneMapper® ID-X.

13.2.3.5 Review of Negative Controls and Reagent Blanks

13.2.3.5.1 Select the negative control sample(s) or reagent blank(s) in the Samples table, then click (Display Plots). Select the "RB & Neg View" Plot Setting from the Plot Settings drop-down list.

13.2.3.5.1.1 The RB & Neg View Plot Setting must be used to view any reagent blank or negative control samples. This plot setting should include the primer dimer range. Alternatively, the Raw Data tab may be used to ensure primer dimer is present.

13.2.3.5.2 Verify that no called peaks are detected in the negative control or reagent blank.

13.2.3.5.2.1 A negative control or reagent blank that has no called peaks but appears to have allelic activity below the analytical threshold, does not render rework.

13.2.3.5.2.2 A negative control or reagent blank exhibiting a single called drop-in peak, with no allelic activity below the analytical threshold, does not render rework.

13.2.3.5.2.3 A negative control or reagent blank exhibiting a single called drop-in peak, with allelic activity below the analytical threshold, does render rework.

13.2.3.5.2.3.1 The associated sample(s), controls, and reagent blank(s) must be re-injected, re-plated and re-injected, re-amplified, and/or re-extracted to resolve the issue.

13.2.3.5.2.4 A negative control or reagent blank exhibiting more than one called peak renders rework.

13.2.3.5.2.4.1 The associated sample(s), controls, and reagent blank(s) must be re-injected, re-plated and re-injected, re-amplified, and/or re-extracted to resolve the issue.

13.2.3.6 Review of Evidence Sample Data

13.2.3.6.1 After all quality control samples have been reviewed and passed, each of the evidence samples should be reviewed. The GeneMapper® ID-X Software provides information on peak quality (height, base pair size, shape, number) as well as designating alleles.

13.2.3.6.2 Select the evidence sample to review in the Samples table, then click (Display Plots).

13.2.3.6.3 Select "Data Interpretation' from the Plot Settings drop-down list.

13.2.3.6.4 Check for true Off Ladder (OL) alleles in the evidence sample profile. A true OL allele is an allele believed to be a true/real allele and not the result of an artifact.

13.2.3.6.4.1 If a true OL allele is thought to be present, reamplify the sample to confirm the OL allele.

13.2.3.6.5 Check for the presence of allele drop-out.

13.2.3.6.5.1 Drop-out can indicate the need to reamplify the sample at a higher amplification target.

13.2.3.6.5.1.1 If the sample was amplified to its maximum target (i.e., a normalization dilution was not performed on the sample), reamplification will not be helpful. In these instances, the analyst should determine if re-extraction is appropriate.

13.2.3.6.6 Consider data RFUs and the stochastic threshold (1400 RFU).

13.2.3.6.6.1 If data RFUs are at, around, and/or below the stochastic threshold, the sample should be re-amplified at a higher target, if possible. Saturation of the 3500 CCD camera should be considered in this decision.

13.2.3.7 Review of Casework Reference Sample Data

13.2.3.7.1 Select the reference sample to review in the Samples table, then click (Display Plots).

13.2.3.7.2 If not already done, select 'Data Interpretation' from the Plot Settings drop-down list.

13.2.3.7.3 Check for true Off Ladder (OL) alleles in the reference sample profile. A true OL allele is an allele believed to be a true/real allele and not the result of an artifact.

13.2.3.7.3.1 If a true OL allele is thought to be present, reamplify the sample to confirm the OL allele.

13.2.3.7.4 Check for the presence of allele drop-out.

13.2.3.7.4.1 Drop-out can indicate the need to reamplify the sample at a higher amplification target.

13.2.3.7.5 If there is indication of male DNA in the sample (presence of Y allele at Amelogenin), check for called allele(s) at DYS391.

13.2.3.7.5.1 If there is reason to suspect drop-out at DYS391, reamplify the sample at a higher amplification target.

13.2.3.7.6 Review any apparent homozygous alleles.

13.2.3.7.6.1 If any apparent homozygous allele is < the stochastic threshold, reamplify the sample at a higher amplification target to increase the allele RFU to ≥ the stochastic threshold.

13.2.3.7.6.1.1 If after re-amplifying the reference sample to its maximum target, the apparent homozygous allele(s) remains < the stochastic threshold, the analyst should re-extract the reference sample. If re-extraction is not possible and/or the issue is not resolved after re-extraction and re-processing, the DNA Technical Leader must be consulted to determine the best course of action.

13.2.3.8 Artifacts

13.2.3.8.1 During the review steps previously described, the analyst will also identify and characterize artifacts.

13.2.3.8.1.1 Some called data may not represent actual alleles that originate in the sample. It is therefore necessary, before the STR typing results can be used for comparison purposes, to identify any potential non-allelic peaks (i.e., artifacts). Artifacts may be PCR products (e.g., stutter and incomplete nucleotide addition), instrumental artifacts (e.g., spikes and raised baseline), or instrumental limitations (e.g., incomplete spectral separation resulting in pull-up). Generally, artifacts such as stutter, incomplete nucleotide addition, and pull-up are reproducible; spikes and raised baseline are generally non-reproducible.

13.2.3.8.1.2 If an artifact can be confidently characterized the sample may be used for analysis. If an artifact cannot be confidently characterized the data will be further assessed through reanalysis (i.e., re-injected, re-amplified, re-quantified, and/or re-extracted).

13.2.3.8.1.2.1 If an allele is modified, re-labeled, and/or deleted within the GeneMapper® ID-X Software this must be documented within the case file.

13.2.3.8.2 Below are examples of artifacts that can be observed in the data:

13.2.3.8.2.1 Stutter:

13.2.3.8.2.1.1 Stutter is believed to be a result of slippage of the polymerase during amplification. This is represented as a small peak, generally one repeat unit lesser than or greater than the true peak.

13.2.3.8.2.1.2 Stutter may also appear as multiples of the repeat unit (e.g., n - 8, n + 8) or portions of the repeat unit (e.g., n – 2, n+2, n-3, n+3).

13.2.3.8.2.1.3 GeneMapper® ID-X Software is programmed to detect and filter (i.e., not call as an allele) n-4 and n+4 stutter peaks using the maximum stutter percentage observed between the MNPD-CL in-house validation and the manufacturer recommendation. These maximum stutter percentages (i.e., stutter ratio thresholds) are defined in the GeneMapper® ID-X Panel Manager.

13.2.3.8.2.1.3.1 The forward stutter (n + 4) ratio threshold is set globally at 4.5% for all loci except D22S1045 which will use the manufacturer forward stutter filter of 8.6%.

13.2.3.8.2.1.4 The forward stutter for Penta E and Penta D will be calculated manually as the forward stutter filter is only applied to the tetra repeats. The forward stutter ratio is 4.5% for Penta E and Penta D.

13.2.3.8.2.1.5 See the charts below for the stutter ratio thresholds.

13.2.3.8.2.1.6 Combination stutter is defined as a stutter allele resulting from the combination of minus (n-2, n-3, n-4, and/or n-8) and plus (n+2, n+3, n+4, and/or n+8) stutter.

13.2.3.8.2.1.6.1 For example, combination stutter could be observed when alleles differ by two repeat units with an allele in between them that differs by one repeat unit (e.g., 12 and 14 alleles with an allele at the 13 position resulting from minus stutter from the 14 allele and plus stutter from the 12 allele). Additionally, combination stutter could result from the combination of different stutter types. For example, a true allele's (e.g., 6) n+8 stutter and a different true allele's (e.g., 9) n-4 stutter could combine (e.g., 8) to create a combined stutter allele. The examples listed are only examples, and not rules, combination stutter can be observed in various ways by combining multiple stutter types.

13.2.3.8.2.1.7 If an allele in this combined stutter position does not meet the minus and/or plus stutter thresholds, the following calculation should be performed to determine if the allele can be characterized as stutter.

13.2.3.8.2.1.7.1 Terms

13.2.3.8.2.1.7.1.1 Allele A – first allele (12 allele in the example above).

13.2.3.8.2.1.7.1.2 Allele B – possible stutter allele (13 allele in the example above).

13.2.3.8.2.1.7.1.3 Allele C – last allele (14 allele in the example above).

13.2.3.8.2.1.7.2 Formula (□ + □ = □)

13.2.3.8.2.1.7.2.1 X = (RFUs of Allele A * maximum plus stutter % of locus)

13.2.3.8.2.1.7.2.2 Y = (RFUs of Allele C * maximum minus stutter % of locus)

13.2.3.8.2.1.7.2.3 Z = Maximum RFU threshold of Allele B (this value will be truncated)

13.2.3.8.2.1.7.3 If RFU of Allele B ≤ Z

13.2.3.8.2.1.7.3.1 Allele B will be characterized as stutter and must not be used in interpretation and/or comparison.

13.2.3.8.2.1.7.4 If RFU of Allele B > Z

13.2.3.8.2.1.7.4.1 Allele B is considered a true peak.

13.2.3.8.2.1.8 After filtering, if a peak(s) is suspected to be elevated stutter (i.e., a true stutter peak whose ratio has exceeded the threshold) the data will be further assessed through reanalysis (i.e., re-injected, re-amplified, re-quantified, and/or re-extracted).

13.2.3.8.2.2 Pull-up:

13.2.3.8.2.2.1 This type of artifact is caused by poor spectral separation of the dye channels and is typically caused by high quantity of input DNA.

13.2.3.8.2.2.2 If pull up peaks are due to poor color separation and not excessive peak height in other channels, then a new spectral calibration may need to be performed and the sample analyzed using the new spectral calibration.

13.2.3.8.2.3 Incomplete +A nucleotide addition (-A):

13.2.3.8.2.3.1 The PCR process utilizes a polymerase that is optimized to add an additional Adenosine nucleotide onto the extended fragment. Excessive input DNA can make this process less efficient resulting in PCR fragments that are one nucleotide shorter than the true amplicon size (-A).

13.2.3.8.2.3.2 Spikes:

13.2.3.8.2.3.2.1 Spikes are peaks that generally have a sharp, needle-like appearance. Commonly, spikes will be observed in most or all of the dye colors, and generally at the same base pair location. Occasionally, single color spikes may also be observed.

13.2.3.8.2.3.3 The software uses a proprietary algorithm that detects spikes based on the peak morphology and will automatically flag spikes within the analyzed range. Occasionally, the software may mislabel a spike as an allele.

13.2.3.8.2.4 Dye Blob/Raised Baseline:

13.2.3.8.2.4.1 This type of artifact is generally caused by free dye-labeled primers that fall within the analyzed range. This results in an elevation of the baseline sometimes beyond the analytical threshold but not generally with any defined or identifiable morphological shape.

13.2.3.8.3 The below charts may be used to evaluate the presence of stutter and possible kit artifacts:

	Stutter Peaks				Other Artifacts
Locus	**In-House (Avg+3)**	**Max (if > Avg+3)**	**Manufacturer (if > Avg+3)**	**n-1**	**Additional**
AMEL	n/a	n/a	n/a	X	
D3S1358	12.1%				
D1S1656	13.3% (n-4) 3.5% (n-2)		14.2% (n-4) 3.6% (n-2)		
D2S441	7.7%		9.2%		
D10S1248	11.6%		12.4%		
D13S317	9.6%		9.8%		
Penta E	5.7%		7.6%		
D16S539	11.0%				84 bases (allele 5)
D18S51	16.1%	17.7%			214 bases
D2S1338	14.9%	19.0%			247 bases
CSF1PO	9.9%				
Penta D	3.4%		6.8%		
THO1	5.6%	7.2%			*71-73 and *75-77 bases (*may call as OL)
vWA	13.7%				
D21S11	14.1%	19.7%			
D7S820	10.6%	12.3%			
D5S818	12.5%	17.1%			
TPOX	6.4%				
DYS391	8.5%		8.7%		
D8S1179	11.5%				
D12S391	20.1%				
D19S433	10.6%	11.6%			
FGA	14.0%	15.9%			
D22S1045	18.7% (n-3)				

Dye Channels	Kit Artifacts
fluorescein (blue)	62-65, 63-68, and -86 bases
JOE (green)	68-71, 79-80, 214, and 247 bases
TMR-ET (yellow)	58-61, 64-67, and 69-72 bases
CXR-ET (red)	58-65 bases

Additional Stutter Thresholds						
	N-2*	**N+2**	**N-8**	**N+8**	**N-3**	**N+3**
All loci	1.9%	2.6%	2.8%	1.5%		
D12S391					2.2%	2.3%
*For N-2, D1S1656 will adhere to percentage in the previous chart.						

13.2.3.8.4 Labeling of Artifacts:

13.2.3.8.4.1 For analysis of sample data, all electropherograms must be visually evaluated by the analyst to ensure that artifacts are not mislabeled. If a peak is mislabeled, the allele call should be edited by the analyst. Care must be taken when evaluating artifacts observed in samples arising from DNA from more than one individual; if necessary, re-injection, re-amplification, re-quantification, or re-extraction may be needed to make a determination of artifact vs. true allele. When a peak has been determined to be a true artifact, the allele should be deleted.

13.2.3.8.4.2 To edit an allele call, select the peak(s) of interest by right clicking on it.

13.2.3.8.4.3 Select Delete Label(s) from the drop-down list.

13.2.3.8.4.3.1 **NOTE**: When multiple peaks are selected at the same time, the changes will be applied to all selected peaks and the same Reason For Change will be applied to all of them.

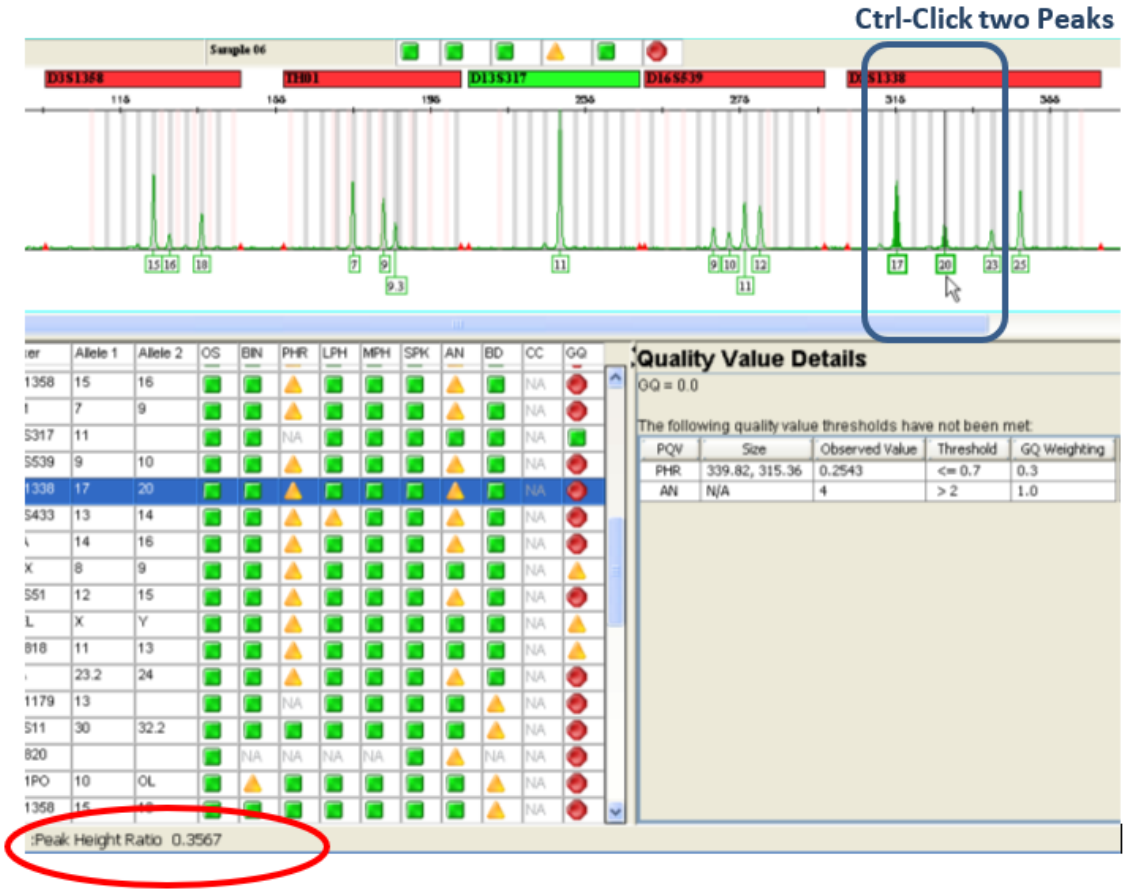
13.2.3.8.4.3.2 When deleting a software labeled peak, indicate Reason For Change (i.e., ART = artifact).

13.2.3.8.4.3.3 The modification, re-labeling, and/or deletion of an allele within the GeneMapper® ID-X Software must be documented within the case file.

13.2.3.9 Peak Height Ratio (PHR):

13.2.3.9.1 The GeneMapper® ID-X Software has a quality flag that can aid in analysis and interpretation of single source and mixed samples based on the peak height ratio of alleles within a locus (PHR PQV). A flag is displayed when there are two alleles present and the ratio between the lower allele height and the higher allele height is below a certain level (60% set in the Peak Quality tab of the analysis method). The ratio is based on the peak height of the called allele peaks.

13.2.3.9.2 In a mixed source sample, it is also possible to ctrl-click on the two allele peaks within the Sample plot then the software will automatically calculate a peak height ratio (PHR) value for the selected peaks and displays the results in the status bar at the bottom of the Samples plot.



13.2.3.9.2.1

13.2.3.10 Off-scale Data (OS):

13.2.3.10.1 When an excess of DNA is added to the amplification reaction, the result can be PCR product that produces a fluorescent intensity that exceeds the dynamic range for detection by the instrument called off-scale data. This is represented in the software by a bright pink line through the data that exceeds the detection threshold of the instrument (i.e., RFUs ≥ 30,000).

13.2.3.10.2 When data is off-scale, peak heights may not be accurately scaled, interfering with data interpretation. Evidence samples with data over 30,000 RFUs will be re-run (i.e., diluted and re-amplified at a lower target). Standards and positive controls with data

over 30,000 RFUs whose artifacts, if any, can be attributed to pull-up may be used without further re-analysis. Standards and positive controls with artifacts not attributable to pull-up will be re-run.

13.2.3.11 Off Ladder (OL) Allele Calls:

13.2.3.11.1 If a peak falls outside of one of the defined bins, the software labels it OL (Off Ladder). OL calls may be caused by the presence of a microvariant allele at a particular locus or sample migration anomalies and artifacts.

13.2.3.11.2 After reamplification to confirm the presence of a called OL, allele names may be assigned to these OL peaks based on the number of complete or partial base pair repeat units.

13.2.3.11.2.1 To evaluate an OL allele call in GeneMapper® ID-X, highlight the sample in question and allelic ladder(s) under the Samples tab.

13.2.3.11.2.2 Click  (Display Plots) and zoom into the peak in question. This should also magnify the ladder(s) automatically.

13.2.3.11.2.3 Compare the base pair size of the peak in question (OL) to, at minimum, the surrounding known ladder allele(s) to determine the size of the peak.

13.2.3.11.2.3.1 If the allele is seen to the right of the largest allelic ladder peak of the locus, it will be assigned the type of the largest allele of the allelic ladder with a greater than sign (>).

13.2.3.11.2.3.2 If the allele is seen to the left of the smallest allelic ladder peak of the locus, the allele will be assigned the type of the smallest allele of the allelic ladder with a less than sign (<).

13.2.3.11.2.3.3 If an allele is seen between two loci and either the locus to the right OR left of the peak contains two peaks, the allele will be considered to belong with the locus not containing two peaks. Naming should follow the directions stated above.

13.2.3.11.2.3.4 If an allele is seen between two loci and neither the higher nor lower molecular weight loci contain an allele or two alleles, the base pair size for the allele in question must be assessed. Compare the base pair sizes of the largest/smallest surrounding allelic ladder peaks to determine which is closest in proximity and falls within an appropriate size distance from the locus. Naming should follow the directions stated above.

13.2.3.11.2.3.5 Alleles that fall between two allelic ladder peaks of the same locus are considered microvariant alleles. Microvariant alleles contain a partial repeat and are designated by a decimal followed by the number of bases in the partial repeat (i.e., an FGA 26.2 allele).

13.2.3.11.2.4 Once the size of the peak and appropriate naming for the OL allele have been determined, the peak should be relabeled by right-clicking on the peak, then selecting "Rename Allele Label" from the drop-down list.

13.2.3.11.2.5 In the "Add Custom Allele Label" dialog box, enter the allele name, then click "OK". Enter the reason for the change in the prompt box.

13.2.3.11.2.5.1 In the prompt box, the bp size of the peak should be noted.

13.2.3.11.2.6 For the case file, select the sample containing the microvariant and one ladder. Zoom to the microvariant locus in the "Microvariant" plot setting. Print to PDF and include with the electropherogram of the used data.

13.2.3.12 Review of Raw Data

13.2.3.12.1 To troubleshoot any anomalies, the causes of poor size-calling, and to determine the start and stop points of analysis, it can be beneficial to examine the unseparated raw fluorescent data.

13.2.3.12.2 In the Project window, click + to expand the Samples folder, highlight each sample to view its associated information.

13.2.3.12.3 The Info tab for the selected sample is displayed. Review the sample-specific information presented in this tab, including error messages, run information, and data collection settings.

13.2.3.12.4 Select the Raw Data tab in the content pane to help evaluate any anomalies, the causes of poor size-calling, and to aid in troubleshooting the start and stop points for analysis.

13.2.3.12.5 Select the EPT Data tab in the content pane. The EPT plot is displayed for the selected sample.

13.2.3.12.6 Select the Project node in the navigation pane to return to the Samples table view.

13.2.3.12.7 To view a sample, highlight the sample then click  (Display Plots).

13.2.3.13 Export of GeneMapper project

13.2.3.13.1 Under the "Tools" tab, select "GeneMapper™ ID-X Manager".

13.2.3.13.2 Enter the associated GeneMapper project name in the “Find Name Containing” field.

13.2.3.13.3 Highlight the project in the list and select “Export...”.

13.2.3.13.4 Browse to the designated export location following the path: This PC > Crime Lab Backup (G:) > Instrument-DNA > GM projects > YYYY “Casework” > Month. Save as a “.ser” file.

13.2.3.13.5 Click “Done” once export is complete.

13.2.3.13.6 Project will remain in GeneMapper until review is complete.

13.2.4 Limitations

13.2.4.1 Not applicable.

13.2.5 References

13.2.5.1 GeneMapper® ID-X Software Version 1.0 Getting Started Guide (October 2007). Applied Biosystems.

13.2.5.2 GeneMapper® ID-X Software Version 1.2 Reference Guide (October 2007). Applied Biosystems.

13.2.5.3 GeneMapper® ID-X Software Version 1.2 (December 2009). Applied Biosystems.

13.2.5.4 GeneMapper® ID-X Software Version 1.3 (October 2011). Applied Biosystems.

13.2.5.5 PowerPlex Fusion System Technical Manual (October 2012). Promega, Madison, WI.

13.2.5.6 GeneMapper® ID-X Software Version 1.4 (December 2012)

13.2.5.7 PowerPlex Y23 System Technical Manual (July 2012). Promega, Madison, WI.

14. Data Interpretation

14.1 STR Data Interpretation

14.1.1 Scope

14.1.1.1 To describe the process of analysis and interpretation of STR data for the purpose of comparison, reporting and statistical analysis.

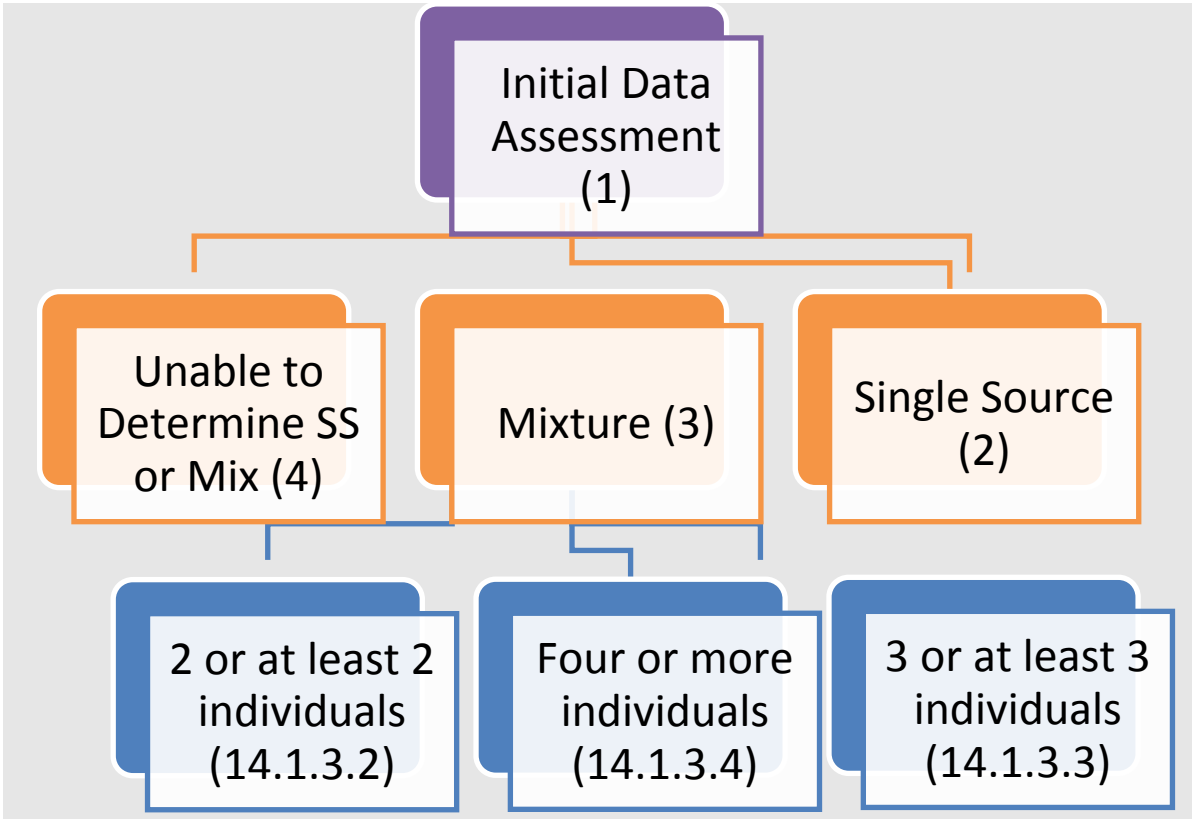
14.1.2 Equipment/Materials/Reagents

14.1.2.1 GeneMapper® ID-X Software v1.6

14.1.2.2 DNA Batch Workbook

14.1.3 Procedure

14.1.3.1



14.1.3.2 (1) Perform an initial assessment of the data to determine if the data represents a single source DNA profile or a mixed DNA profile. In order to make this assessment, peaks must be detected at 5 or more locations.

14.1.3.3 (2) A DNA profile may be considered as having originated from a single individual if no more than two peaks are observed at each locus and the balance of the alleles within a locus is approximately the same. All loci should be taken into account when making this decision.

14.1.3.3.1 In rare instances, a tri-allelic pattern may be observed. A confirmed tri-allele would not forfeit the assessment of the profile as a single source.

14.1.3.3.2 Although average peak height ratios for a single source sample at a heterozygous locus are expected to be above 60% when DNA quantity is above stochastic levels (as demonstrated in the MNPD-CL validation data), heterozygote balances have been observed below 60% for single source samples even at sufficient quantities of input DNA. The peak height ratios observed in the positive control may be used to assess expected peak height ratios for samples amplified at the target amount used for the positive control. Samples amplified below the target amount and mixtures may not meet the expected peak height ratios observed in the positive control. See the Table below from the MNPD internal validation "Validation of Promega PowerPlex® Fusion using a Veriti Thermal Cycler and 3500 Series Genetic Analyzer Validation Report".

14.1.3.3.3

Run 2 Instr #2 Comparison											
Input Target	APH	APH stdev	APH %CV	Max PH	Min PH	[redacted]	avg PHR	PHR stdev	PHR %CV	Min PHR	[redacted]	# of alleles
0.0036ng	68	83	123%	285	0		72%	24%	36%	62%		14
0.0072ng	135	130	97%	445	0		67%	20%	32%	43%		23
0.0156ng	223	168	77%	732	19		64%	22%	36%	30%		32
0.0313ng	527	274	52%	1330	119		61%	22%	37%	22%		41
0.0625ng	1030	480	47%	2467	408		65%	21%	33%	27%		43
0.125ng	2400	806	34%	4717	1161		73%	17%	23%	43%		44
0.25ng	4789	1622	34%	10122	2355		82%	13%	16%	54%		44
0.5ng	8174	2729	34%	16027	4442		84%	11%	13%	61%		44
1ng	15338	3813	25%	23632	9503		87%	8%	9%	70%		44
2ng	20750	6741	32%	31255	7217		92%	8%	9%	72%		44

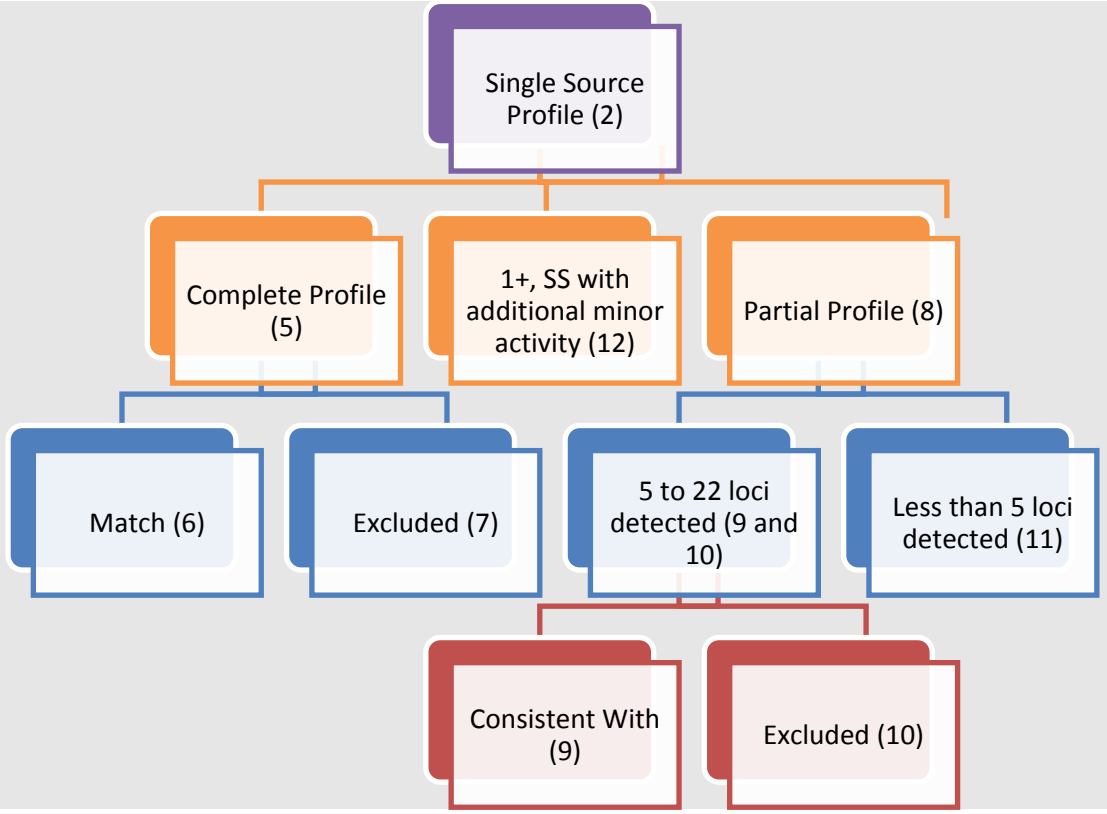
14.1.3.4 (3) The source of a DNA sample may be from more than one person. A sample may be considered a mixture of DNA from more than one individual if the sample contains three or more peaks at two or more loci and/or the difference in peak height ratio for several loci is below the established 60% balance. All loci should be taken into account when making this determination.

14.1.3.4.1 If the data is determined to represent a mixture of DNA profiles from individuals, the number of individuals or the minimum number of individuals may be assumed. If all of the DNA data for the sample is below the stochastic threshold, no assumptions will be made regarding number of contributors/minimum number and no comparisons will be performed. Observations of potential activity below AT may prevent the analyst from assuming an exact number of contributors and/or using minor data.

14.1.3.5 (4) If the data is too limited (in RFU's and/or loci) to determine whether the data represents a single source or mixture, no determinations should be made on the data. Data should be present at a minimum of 5 loci in order to make this determination. Observations of potential activity below AT may also prevent the analyst from assessing SS or mixture.

14.1.3.6 Single Source Profile Informational Flowchart

14.1.3.6.1



14.1.3.6.2 SS Profile Flowchart Information

14.1.3.6.2.1 (5) A profile may be considered complete when a genotype can be concluded for all autosomal STR loci.

14.1.3.6.2.1.1 **TECHNICAL NOTE**: To indicate the gender of the contributor of a particular biological sample the amelogenin locus may be used. A biological sample exhibiting a single peak above stochastic threshold at approximately 89 bp (X allele) and no peak at DYS391 will generally be considered to have originated from a genetically female individual. A biological sample exhibiting a peak at approximately 89 bp (X allele) and a peak at approximately 95 bp (Y allele) with the presence of a peak at DYS391 will generally be considered to have originated from a genetically male individual.

14.1.3.6.2.2 (6) If a known sample is available for comparison, the same DNA information in the Q and K will be reported as matching one another. RMP or modified RMP (mRMP) will

be calculated; refer to 15.0 of the Forensic Biology Technical Procedures Manual for more information on mRMP.

14.1.3.6.2.3 (7) The known sample (if available) will be deemed excluded as the source of the questioned sample if the DNA profile from the known sample is different from the DNA profile of the questioned sample.

14.1.3.6.2.4 (8) A profile will be deemed partial when no peaks is detected at Amelogenin or one or more autosomal STR loci.

14.1.3.6.2.4.1 **TECHNICAL NOTE**: The stochastic threshold (STO) can be used as a predictor for allelic drop-out. The stochastic threshold is 1400rfu. Alleles detected below this threshold may be missing their sister allele. If the alleles are above this threshold, generally, both sister alleles can be presumed to be present. If a locus, in an assumed single-source sample, exhibits two peaks, one or both of which are below the stochastic threshold, the analyst can conclude the locus to be heterozygous and calculate the genotypic frequency for statistics. If a locus, in an assumed single-source sample, exhibits one peak which is below the stochastic threshold, the analyst may still use the locus for comparison purposes and calculate statistics using 2p in the event of a match (refer to 15.0 of the Forensic Biology Technical Procedures Manual for more information on 2p).

14.1.3.6.2.5 (9) If a known sample is available for comparison and a minimum of 5 loci are detected in the questioned sample, consistent DNA information in the Q and K will be reported as consistent with each other. RMP or modified RMP (mRMP) will be calculated; refer to 15.0 of the Forensic Biology Technical Procedures Manual for more information on mRMP.

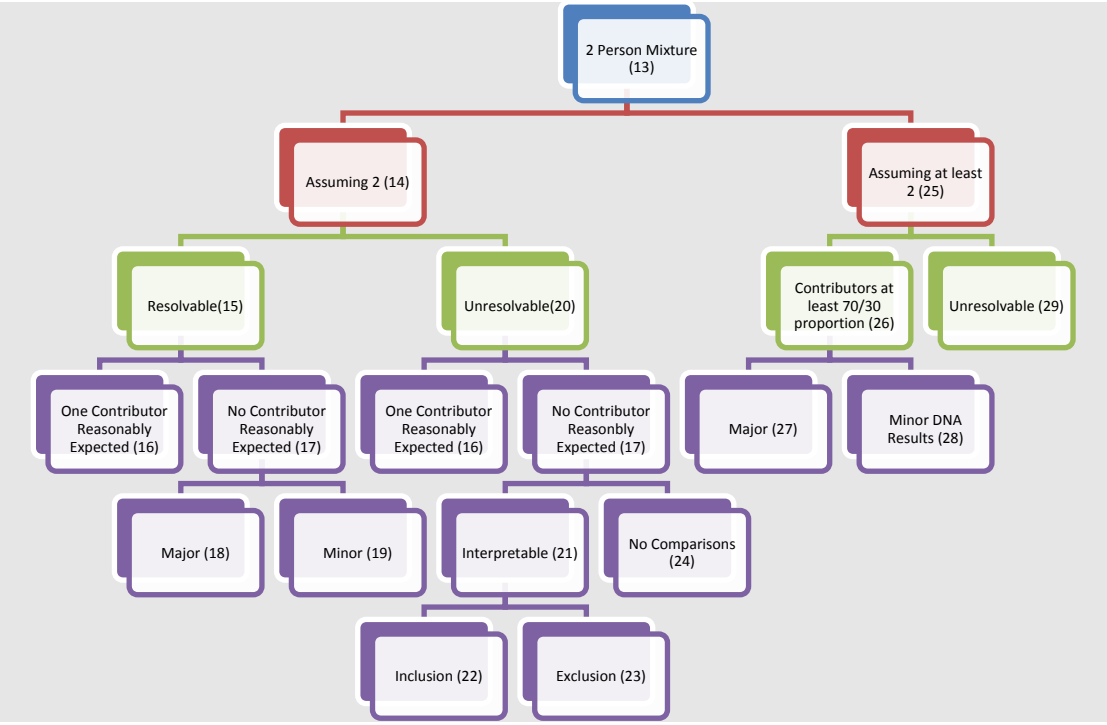
14.1.3.6.2.6 (10) The known sample (if available) will be deemed excluded as the source of the questioned sample if the DNA profile of the known sample is different from the partial DNA profile of the questioned sample, taking into account the possibility of drop-out, drop-in, and artifact interference/outliers.

14.1.3.6.2.7 (11) When DNA information is obtained at less than 5 loci, no comparisons to the data will be performed.

14.1.3.6.2.8 (12) A DNA profile is deemed 1+, when 1) one additional minor activity (not in artifact position) at one locus OR 2) limited minor activity is present in locations of possible artifact interference, is detected in addition to a single source interpretable profile. This does not include instances of confirmed tri-allelic patterns.

14.1.3.7 Mixture Profile Informational Flowchart (2 Person Tree)

14.1.3.7.1



14.1.3.7.2 Mixture Profile Flowchart Information (2 Person Tree)

14.1.3.7.2.1 (13) A mixture of DNA that comes from two (2) individuals is generally indicated by not more than four (4) alleles at any locus. At loci where four (4) alleles are present, phr should be evaluated based on validation data.

14.1.3.7.2.1.1 **TECHNICAL NOTES**: When an assumption of any number of contributors is made, the number of alleles and/or peak height ratio present at any locus should be taken into account to determine the possible reasonable genotypic combinations for the contributors. If allele(s) fall below the stochastic threshold, these alleles may still be used when determining these combinations. If the genotype of a contributor cannot be fully resolved, the analyst may employ the use of 2p for statistical calculation (refer to 15.0 of the Forensic Biology Technical Procedures Manual for more information on 2p).

14.1.3.7.2.2 (14) When the data obtained reflects the presence of only two contributors and no more than two contributors, an assumption can be made for interpretation of the profile. When this assumption is made, it must be reported. The entire profile should be considered when making an assumption of the number of contributors including,

but not limited to, the maximum number of alleles at the loci, peak height ratios of possible sister alleles, and possible artifact interference.

14.1.3.7.2.3 (15) The mixture should support at least a 70/30 proportionate contribution of the major to minor donor in order to deduce a major and minor profile.

14.1.3.7.2.4 (16) **A contributor is reasonably expected if at least one of the following apply:**

14.1.3.7.2.4.1 The sample is an intimate sample from the known individual (A sample will only be deemed intimate in nature when it originates directly from the donor's body [i.e., fingernail clippings/swabbing, vaginal swabs, oral swabs, or a swabbing from any skin surface]).

14.1.3.7.2.4.2 The sample was obtained from clothing worn by the known individual. The clothing must be collected from the individual's body or identified by the individual as their clothing). Documented communication stating this must be maintained (i.e., MNPD 282 form, phone log, email, evidence label, etc.).

14.1.3.7.2.4.3 The sample is collected from an area/location or item that the individual is known to have occupied or touched (i.e., vehicle, residence, etc.). Documented communication stating this must be maintained (i.e., MNPD 282 form, phone log, email, evidence label, etc.).

14.1.3.7.2.4.4 If a DNA profile was developed from a sample that qualifies for at least one of the above, a foreign DNA profile may be determined. This can be determined by "backing out" the reasonably expected donor's DNA profile. In order to "back out" the known contributor's profile, the DNA data must be interpretable at a minimum of 5 loci. Once the reasonably expected donor is determined to be included in the DNA data, the individual's profile is assumed to be present and may be "backed out" of all loci with allelic activity above AT. The assumption will be reflected in the report. The remaining alleles can then be attributed to the foreign contributor, taking into consideration potential artifact interference.

14.1.3.7.2.4.5 It should also be noted that the known contributor and the foreign contributor can share alleles. Percent contribution and PHR can be used to aid in determination of genotype of the foreign individual in these situations. If the foreign donor is assessed as the minor contributor (equal to or less than 30% of the RFU's detected) and exhibits only one obligate allele that occurs below the stochastic threshold, the allele, any (allele +) should be assigned.

14.1.3.7.2.4.6 Deduced foreign profiles will then proceed to (5)Complete profile or (8)Partial profile of the 14.1.3.1 Single Source Profile Information Flow Chart.

14.1.3.7.2.5 (17) Samples that do not meet one of the criteria listed in (16) will be further classified as Deduced major and minor profiles.

14.1.3.7.2.6 (18) The major DNA profile will be considered interpretable when the major contributor can be determined at a minimum of 5 loci. A restricted major contributor will not be determined at locations where all allelic activity occurs below stochastic threshold. Instead, possible allele combinations may be used at these locations, provided that both contributors are at least partially represented at these locations. At locations where artifacts or potential allele sharing may occur, the analyst should consider if the activity would drop both donors below stochastic threshold. To consider a pair of alleles heterozygous for the major contributor, the alleles must be a minimum of 60% balanced. The approach utilizing possible allelic combinations may also be used at locations where 60% peak height balance is not exhibited or the major/minor becomes ambiguous in distribution. The deduced major profile will be determined prior to comparison with known DNA profiles.

14.1.3.7.2.6.1 **TECHNICAL NOTE**: The major contributor may be deduced at loci where the minor contributor may not be represented, provided that all alleles present at this location are not below stochastic threshold. If the locus for which the major is being deduced exhibits only two peaks, at least one of those peaks must be above 1400rfu. For locations, exhibiting only one peak, the single peak must be above 2800rfu.

14.1.3.7.2.6.2 Deduced restricted major profiles will then proceed to (5)Complete profile or (8)Partial profile of the 14.1.3.1 Single Source Profile Information Flow Chart.

14.1.3.7.2.7 (19) Where a minor profile can be resolved, this minor profile must have data at a minimum of 5 loci in order to use for comparison (refer to the SS Profile Flowchart following a partial profile for more information), taking into consideration potential artifact interference.

14.1.3.7.2.7.1 Minor activity at locations with possible artifact interference can be omitted from comparison. Reasons for the omission must be documented within the case file. Locations omitted from comparison based on this reason will be determined prior to comparison to known contributors.

14.1.3.7.2.7.2 Deduced restricted minor profiles will then proceed to (5)Complete profile or (8)Partial profile of the 14.1.3.1 Single Source Profile Information Flow Chart.

14.1.3.7.2.7.3 If combinations of allelic activity were used to deduce the major contributor (See (18) above), the same approach may be used to express the potential minor contributor. See (22) and (23) below for information on comparison of DNA profiles from known samples to unrestricted major/minor evidence profiles.

14.1.3.7.2.8 (20) A profile assumed to contain only two contributors will be deemed unresolvable when the two contributors to the mixture do NOT demonstrate at least a 70/30 proportional difference.

14.1.3.7.2.9 (21) The unresolvable data is deemed interpretable when the assumed number of individuals is at least partially represented at a minimum of 5 loci (i.e., at least 3 or 4 alleles and/or PHR's of two alleles inconsistent with validation data), taking into consideration potential artifact interference. Minor activity at these locations may be omitted from comparison due to possible artifact interference. Reasons for the omission must be documented within the case file. Alleles omitted from comparison based on artifact interference and the possible allele combinations at interpretable locations will be determined prior to comparison to known contributors.

14.1.3.7.2.9.1 Once the interpretable loci have been determined, probable allelic combinations will be determined and documented. Heterozygous combinations meeting a peak height of at least 22% (based on validation) will be considered possible pairs.

14.1.3.7.2.10 (22) When a DNA profile of a known sample is represented in the predetermined allele combinations, the known contributor will be included as a possible contributor to the mixed DNA profile. mRMP will be calculated based on restricting the mixed DNA profile to only 2 individuals. Statistics should only be performed at interpretable loci where the known contributor's allele combinations are represented.

14.1.3.7.2.10.1 **TECHNICAL NOTE**: The stochastic threshold (STO) can be used as a predictor for allelic drop-out. The stochastic threshold is 1400rfu. Alleles detected below this threshold may be missing their sister allele. If the alleles are above this threshold, generally both sister alleles can be presumed to be present; however, if the majority of other loci detected in a profile have alleles below this threshold, it can be an indication that DNA from the low-level donor has dropped out completely.

14.1.3.7.2.11 (23) The known sample (if available) will be deemed excluded as a contributor to the questioned sample if the DNA profile of the known contributor is different from the obtained/deduced DNA profile. Non-concordance at a single locus may not result in an overall exclusion when the difference may be explained by the science (i.e. the possibility of drop-out, drop-in, or artifact interference). In those instances, if the discrepancy can be reasonably attributed to amplification, genetic, or other outliers, the analyst will drop the locus in question from the frequency estimated and proceed with reporting the inclusion. The reasons for dropping the locus should be clearly documented in the examination records.

14.1.3.7.2.12 (24) When the non-resolvable mixed DNA profile does not contain interpretable DNA data at a minimum of 5 loci, no comparisons will be performed.

14.1.3.7.2.13 (25) When the data obtained generally reflects the presence of two contributors but there is the possibility of a third contributor (i.e., additional peak only occurring in potential artifact locations, a 5th peak at one location and/or phr below expected values based on validation at multiple loci), this will be interpreted and reported as a DNA profile containing at least two contributors.

14.1.3.7.2.14 (26) If the mixture supports at least a 70/30 proportionate contribution of one of the contributors, a restricted major profile may be deduced.

14.1.3.7.2.15 (27) Follow step (18) above to deduce the major donor. If the major donor matches/is consistent with an individual that qualifies as reasonably expected, an assumption statement may be used. If the individual does not qualify as one that would be reasonably expected on a sample, proceed to (5) Complete profile or (8) Partial profile of the 14.1.3.1 Single Source Profile Information Flow Chart.

14.1.3.7.2.16 (28) Where minor DNA results are obtained in a mixture in an “at least” situation, no comparisons will be performed.

14.1.3.7.2.17 (29) When the data obtained in the mixture does not demonstrate at least a 70/30 proportional difference, no comparisons will be performed.

14.1.3.8 Mixture of 3 individuals.

14.1.3.8.1 A mixture of DNA that comes from three (3) individuals is generally indicated by no more than six (6) alleles at any locus detected. When the data obtained reflects the presence of only three individuals and no more than three, an assumption can be

made for interpretation of the profile. When this assumption is made, it must be reported.

14.1.3.8.1.1 When the data obtained generally reflects the presence of three contributors but there is the possibility of a fourth (i.e., additional peak only occurring in potential artifact locations, the presence of a 7th peak at one location), this will be interpreted and reported as a DNA profile containing at least three contributors. No comparisons will be performed to this data.

14.1.3.8.1.2 If three contributors are assumed, if an 85/15 proportion is found at all six allele loci, then the major genotype can be determined at these loci. Proportions cannot be calculated or applied to loci containing less than six alleles (i.e., loci with possible allele sharing). A visual assessment of allele RFUs at loci with less than six alleles is not a validated method to determine the major genotype for the remaining loci and will not be performed. The remaining loci (i.e., loci with possible allele sharing) will not be further deconvoluted.

14.1.3.8.1.2.1 If an 85/15 proportion is not found at all six allele loci, then the major genotype cannot be determined, and the profile cannot be deconvoluted. No comparisons will be made to this data.

14.1.3.8.1.3 Interpretation of a mixed minor (or a mixed major) from a three-person mixture has not been validated. Interpretation of the minor donors (or two major donors) of a three-person mixture should not be conflated with the interpretation of a traditional two-person mixture, as they are not the same. Mixed minors (nor mixed majors) will not be deconvoluted. No comparisons will be made to this data.

14.1.3.8.1.4 A reasonably expected individual may be “backed out” of a three-person mixture.

14.1.3.8.1.4.1 If the reasonably expected individual appears to be the major genotype, then 14.1.3.8.1.2 is applicable for the mixed minor.

14.1.3.8.1.4.2 If the reasonably expected individual appears to be a minor genotype, then 14.1.3.8.1.1 is applicable, with the exception that the reasonably expected individual can always be determined. In this instance, no determinations can be made about the third donor (i.e., remaining minor donor).

14.1.3.8.1.4.3 Two reasonably expected individuals may be “backed out” and any remaining alleles will be assigned to the remaining contributor.

14.1.3.9 Mixture of 4 or more individuals

14.1.3.9.1 When the data obtained generally reflects the presence of four or more contributors (represented as greater than 6 alleles at multiple loci), this will be reported as a DNA profile containing at least four contributors. A mixture of 4 or more individuals will generally not be interpreted except in certain situations which would require DNA Technical Leader approval prior to interpretation.

14.1.3.10 Conclusion Statements

14.1.3.10.1 The following terms will be used when comparing a known DNA profile to a questioned/evidence DNA profile in order to make a conclusion.

14.1.3.10.1.1 Match: The question DNA profile is a complete profile and the DNA profile of the known contributor is the same at all loci tested.

14.1.3.10.1.2 Consistent with: The question DNA profile is a partial DNA profile where greater than 5 loci have been detected. The DNA profile of the known contributor is concordant with the DNA profile of the question sample at the loci detected.

14.1.3.10.1.2.1 **TECHNICAL NOTE**: For mixtures where an assumption of a number of contributors are made, the analyst should assess whether all of the DNA typing results obtained in the mixed sample are accounted for when including multiple known samples. The report wording shall accurately reflect this assessment.

14.1.3.10.1.3 Excluded: The known sample (if available) will be deemed excluded as a contributor to the questioned sample if the DNA profile of the known contributor is different from the obtained/deduced DNA profile. Non-concordance at a single locus may not result in an overall exclusion when the difference may be explained by the science (i.e., the possibility of drop-out, drop-in, or artifact interference). In those instances, if the discrepancy can be reasonably attributed to amplification, genetic, or other outliers, the analyst will drop the locus in question from the frequency estimated and proceed with reporting the inclusion. The reasons for dropping the locus should be clearly documented in the examination records.

14.1.3.10.1.4 Inconclusive comparison: An analyst may render a comparison inconclusive. This conclusion should be reserved for data that exhibits poor quality (i.e., degradation, inhibition, peak heights below stochastic threshold, peak height balance less than 60%, etc.). An inconclusive comparison may occur when the non-concordance is observed in activity below stochastic threshold at no more than 20%

of the profile. The non-concordance must be scientifically supported (i.e., elevated artifact, drop-in, drop-out, masked alleles in a deduction, etc.).

14.1.3.10.2 **Note**: For reports issued in the simplified table format, comparison conclusions will be reported by placing the name of the individual in the "Included" or "Excluded" columns.

14.1.4 Limitations

14.1.4.1 When a probative result is obtained for an evidence sample and an inconclusive result is obtained at more than one locus for a known reference sample, the known reference sample will be re-typed or re-amplified in order to produce a complete DNA profile. To the extent possible, complete DNA typing results will be obtained for reference samples. If a partial and/or low level profile from the reference standard must be used for comparison, locations where the analyst is confident that the known contributor is fully represented will be used for comparison. Locations exhibiting a single allele must be above stochastic threshold.

14.1.5 References

14.1.5.1 Internal Validation of Promega PowerPlex® Fusion using a Veriti Thermal Cycler and 3500 Series Genetic Analyzer (August 2014), Sorenson Forensics at MNPD-CL Forensic Biology Unit.

14.1.5.2 Forensic Biology Quality Manual; Section 9.0 – Analytical Procedures

14.1.5.3 Scientific Working Group on DNA Analysis Methods (SWGDAM). "SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories." 14 Jan. 2010. Web. <swgdam.org>.

14.1.5.4 National Research Council. The Evaluation of Forensic DNA Evidence (NRCII). Washington, D.C: National Academy, 1996.

15. Statistical Analysis

15.1 Scope

15.1.1 To describe the process of calculating the statistical frequency of occurrence of evidentiary DNA profiles that have been established as probative in the context of the case. Statistical analysis aids in the assessment of a match or inclusion.

15.2 Equipment/Materials/Reagents

15.2.1 MNPD Statistical Workbook

15.3 Procedure

15.3.1 Formulae

15.3.1.1 The genotype frequency associated with a particular pattern of alleles from a sample is based upon principles of Hardy-Weinberg equilibrium.

15.3.1.2 If a single source sample under analysis demonstrates two different alleles, the genotypic frequency at a particular locus is determined by the equation 2pq, where p and q represent the frequencies of allele #1 and #2.

15.3.1.3 If a single source sample under analysis consists of the same allele, the genotypic frequency at a particular locus is determined by the equation $p2 + p(1-p)\theta$, where $\theta = 0.01$ and p represents the frequency of the allele.

15.3.1.4 For rare alleles, less than or equal to 5 occurrences in the database, the minimum allele frequency will be calculated using $5/2N$ where N is the number of individuals sampled in the three separate ethnic groups (Caucasians, Hispanics, and African-Americans) from the database.

15.3.2 2p Rule

15.3.2.1 For single-alleles that fall below the stochastic threshold, the formula 2p will be applied, instead of p2, where p represents the frequency of the allele. This formula calculates for an “allele plus any” genotype combination in the event the sister allele has dropped out. An assumption of contributors (1, 2, or 3) must be made for the 2p rule to be applied.

15.3.3 Random Match Probability (RMP)

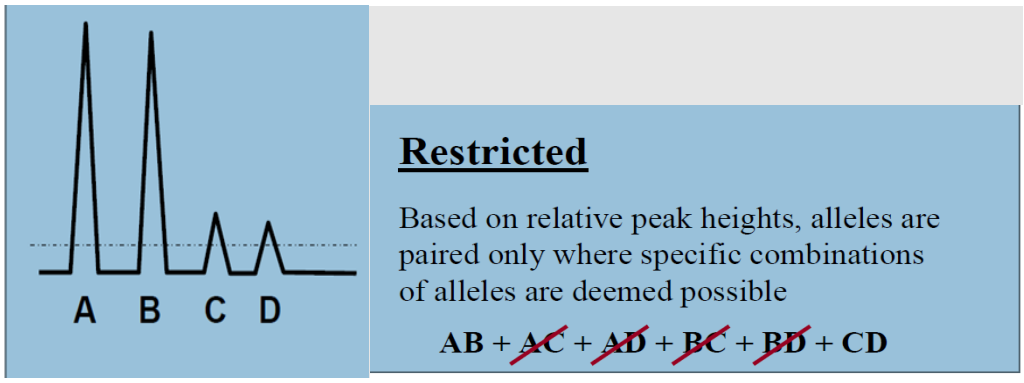
15.3.3.1 A random match probability will be determined for single source samples using the product rule. The product rule may also be applied for a mixture sample when the analyst is able to fully resolve a single profile for a major, minor, or foreign contributor to the mixed sample.

15.3.4 Modified Random Match Probability (mRMP)

15.3.4.1 Modified Random Match Probability (mRMP) is the application of the RMP formula to evidentiary profiles where the number of contributors is assumed. In addition to an assumption of the number of contributors, peak height ratios and mixture ratio assessments will be utilized in the interpretation of the evidentiary profile (See Figure 1 below for an example).

15.3.4.2 When there is more than one genotype being calculated, the mRMP is the sum of the individual frequencies for the determined genotypes at a locus included following a mixture deconvolution.

15.3.4.3



15.3.4.3.1 Figure 1, SWGDAM Interpretation Guidelines 4.

15.3.4.4 In a mixture where alleles are present below the stochastic threshold, the mRMP must account for all heterozygotes and homozygotes represented by the detected alleles. This can be done by calculating using 2p as long as the number of contributors is assumed. However, if there is a reasonable possibility that locus dropout could have led to the loss of an entire genotype, then a statistical calculation should not be performed for that locus. In this scenario, the analyst will assign the value of 1 to the locus (i.e., not use the locus for statistical weight).

15.3.4.5 At minimum, the genetic loci and the assumptions used for statistical calculations must be documented in the case notes.

15.3.5 MNPD Statistical Workbook

15.3.5.1 Analysts can perform the statistical analysis for their samples by hand. They may also use a validated Excel based program capable of calculating both standard RMP and mRMP utilizing the formulae described in the preceding sections of this technical procedure.

15.3.5.2 Open the MNPD Statistical Workbook, complete the case number (noted as "incident" on worksheet), item number, and date fields at the top of the Entry page.

15.3.5.3 Enter the allele calls for the evidentiary sample into the corresponding boxes (Alleles 1 & 2) of the loci. If you are entering a homozygous genotype, you must enter the allele twice (Allele 1 & 2).

15.3.5.4 If multiple genotypic combinations are possible at a locus, utilize locations 1 through 6 for entry of these genotypes. The statistical workbook will calculate the genotypic frequency at each and sum them together to get a combined locus frequency to be applied using the product rule. If the sum of the frequencies is greater than 1, then a value of 1 will be given to the locus.

15.3.5.4.1 **NOTE**: The maximum number of genotypes that can be entered per locus is six.

15.3.5.5 If allelic dropout is possible, the statistical workbook will utilize the 2p calculation when the analyst enters the obligate allele in the first box location (Allele 1) and enters a + in the second box (Allele 2). After entry of the + symbol, press Enter or the Tab key to move to the next cell.

15.3.5.5.1 **NOTE**: Use the Tab key to progress after each allelic or + entry. If you use the mouse to click into another cell within the statistical workbook after entering a +, Excel will treat this as development of a formula. If this occurs, hit Esc to exit.

15.3.5.6 The bottom of the Entry page will display the calculated profile frequencies for the Caucasian, African-American, and Hispanic populations from the Hill et al database also referenced at the bottom of the Entry page. The Entry page should be converted to PDF for collection into the electronic casefile. The most common statistical frequency out of the three that were calculated will be selected for reporting in the final case report.

15.3.5.7 Once this page has been saved as a PDF, the Clear button at the top left corner of the Entry page can be clicked to clear out all of the entered data to prepare the statistical workbook for entry of new data (including case number, item number, and date).

15.4 Limitations

15.4.1 Statistical calculations for the results of each comparison in which a match or inclusion was made must be reported, except when at least one of the following apply:

15.4.1.1 An association is made between the profile derived from an intimate sample and the individual from whom that sample was collected

15.4.1.2 When a differential extraction is performed, a statistic is required for only one fraction of the sample if the profiles, for which a statistic will be applied, are consistent with one another. The presence of the same profile in both fractions is reasonably expected since both extracts originated from the same sample.

15.4.1.3 An association is made between a profile derived from clothing and the individual from which the clothing was worn. The clothing must be collected from the individual's body or identified by the individual as their clothing. Documented communication stating this must be maintained (i.e., MNPD 282 form, phone log, email, evidence label, etc.).

15.4.1.4 The sample is collected from an area/location or item that the individual is known to have occupied or touched (i.e., vehicle, residence, etc.). Documented communication stating this must be maintained (i.e., MNPD 282 form, phone log, email, evidence label, etc.).

15.4.2 Statistical calculations for more than one test may be reported together only if the results of those calculations are identical to each other.

15.5 References

15.5.1 Performance Check of a Statistical Calculations Workbook (December 2014), MNPD-CL Forensic Biology Unit.

15.5.2 ASCLD/LAB Board of Directors Clarification. Special Notice about Reporting DNA Test Results. 5/16/2014.

15.5.3 Hill et al. "Allele Frequency Values for Unrelated U.S. Population Samples (N=1036) at 29 Autosomal STR loci in Commercial STR Multiplex Kits." FSI: Genetics, 7 (2013) e82-e83.

15.5.4 Scientific Working Group on DNA Analysis Methods (SWGDAM). "SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories." 14 Jan. 2010. Web. <swgdam.org>.

15.5.5 Steffen et al. "Corrigendum to 'U.S. Population Data for 29 Autosomal STR Loci' [FSI; Genetics 7(2013)e82-e83]." FSI: Genetics, 31(2017) e36-e40.

15.5.6 National Research Council. The Evaluation of Forensic DNA Evidence (NRCII). Washington, D.C: National Academy, 1996.

16. Forensic Biology Report Writing

16.1 Scope

16.1.1 To describe the process for reporting results from testing items of evidence

16.2 Equipment/Materials/Reagents

16.2.1 Completed Notes, Worksheets, and Interpreted DNA Data

16.2.2 LIMS software

16.3 Procedure

16.3.1 Access the LIMS software and open the case for which the Forensic Biology report will be drafted. Click on the REQUESTS tab. Right click on the request and select RELATED INDIVIDUALS. Highlight all suspects and victims listed on 282(s) in Available Persons. Click the double down arrow button to move the names to the Related Persons field and click OK.

16.3.2 For information in table format

16.3.2.1 Access the LIMS software and open the case for which the Forensic Biology report will be drafted. Click on the EVIDENCE tab. Right click on the parent item for entry of serological results and right click on the child item for the DNA result entry. Verify that the EVIDENCE TYPE has been selected and click on the … button to begin the entry for results.

16.3.2.1.1 Use the - to clear the box. Do not use this as a “not applied” or “not tested” option.

16.3.2.2 Screening Results

16.3.2.2.1 For serology results, do not enter results under the SEROLOGY heading, only enter under the SCREENING heading. Use the dropdown menu to select the results for the item.

16.3.2.2.2 Report serology results under the parent item, along with the metrics for that item. If no serology was performed for the case, leave boxes under SCREENING blank; do not enter N/A. This will ensure that the Serology Table does not show up on the report.

16.3.2.2.3 Table format for SCREENING:

Column Headings	**Detection of Possible Biological Stains:** *[Choose one of the following]*	**Blood:** *[Choose one of the following]*	**SEMEN:** *[Choose one of the following]*	**Forwarded for DNA:** *[Choose one of the following]*
SELECTIONS	Not tested	Not tested	Not tested	N/A
	Stain detected *(ALS positive – absorption or fluorescence)*	Identified *(HemaTrace positive)*	Not identified *(All tests performed negative)*	Yes *(this should only be used when stain/area referenced in previous columns is forwarded to DNA)*
	No stain detected *(ALS negative)*	Not identified *(all tests performed negative)*	Presumptive positive, confirmatory negative *(AP and/or p30 positive*, micro negative)*	No
	INC due to background interference	Presumptive positive, confirmatory negative *(KM positive, HemaTrace negative)*	Presumptive positive, not confirmed *(AP and/or p30 positive*, micro not performed)*	
		Presumptive positive, not confirmed *(KM positive, HemaTrace not performed)*	Sperm identified	
		Presumptive inconclusive, confirmatory negative	Presumptive positive, confirmatory inconclusive	
		Presumptive inconclusive, not confirmed	Presence of sperm inconclusive	

* If only one semen presumptive test is positive, still report as presumptive positive using the paragraph form.

16.3.2.3 DNA Results

16.3.2.3.1 Report DNA results under the child item. All boxes under DNA must have data. Do not leave any boxes empty (at minimum N/A can be entered).

16.3.2.3.2 Table format for DNA:

16.3.2.3.2.1**Note**: All boxes must have an entry (N/A, major, minor, etc.) if the item was tested for DNA. No boxes are to be left blank. If you need to clear results for an item, select - in all boxes for item.

Column Headings	**Y-Screen Results:** *[Choose one of the following]*	**STR Results:** *[Choose one of the following]*	**Contributors Assumed (#):** *[Choose one of the following]*	**Persons Reasonably Expected:** *[free type – must enter text (ie. name or N/A)]*	**Profiles for Comparison:** *[Choose one of the following]*	**Exclusions:** *[Free type – must enter text (ie. name or N/A). If reporting major and/or minor, specify (ie. Major: Name).]*	**Inclusions:** *[Free type – must enter text (ie. name or N/A). If reporting major and/or minor, specify (ie. Major: Name).]*	**Statistic:** *[Free type – must enter text (ie. stat or N/A). If reporting major and/or minor, specify (ie. Major: For XX loci, approximately 1 out of…" Or Major: For XX loci, rarer than 1 out of 100 trillion).]*	**Profiles Uploaded to CODIS:** *[Choose one of the following]*
SELECTIONS	Negative	Mixture	1		Foreign				Not applied *(eligible but not uploaded, another sample chosen)*
	No determinations	No determinations	1 with possible additional contributor		Major & minor				Not eligible

Column Headings	**Y-Screen Results:** *[Choose one of the following]*	**STR Results:** *[Choose one of the following]*	**Contributors Assumed (#):** *[Choose one of the following]*	**Persons Reasonably Expected:** *[free type – must enter text (ie. name or N/A)]*	**Profiles for Comparison:** *[Choose one of the following]*	**Exclusions:** *[Free type – must enter text (ie. name or N/A). If reporting major and/or minor, specify (ie. Major: Name).]*	**Inclusions:** *[Free type – must enter text (ie. name or N/A). If reporting major and/or minor, specify (ie. Major: Name).]*	**Statistic:** *[Free type – must enter text (ie. stat or N/A). If reporting major and/or minor, specify (ie. Major: For XX loci, approximately 1 out of…" Or Major: For XX loci, rarer than 1 out of 100 trillion).]*	**Profiles Uploaded to CODIS:** *[Choose one of the following]*
	Not applied	No result *(No data above AT)*	2		Major only, minor data too limited				One time search only
	Positive	Not tested	3		Mixed foreign				Yes
	Positive/NFT	Single source	4+		Mixture				Yes – major & minor
		Stop at quantitation	At least 2		N/A				Yes – major & partial minor
			At least 3		No foreign				Yes – major only
			N/A		None – Data too limited				Yes – partial
			No determinations		None – Data too complex				Yes – partial major & minor

Column Headings	**Y-Screen Results:** *[Choose one of the following]*	**STR Results:** *[Choose one of the following]*	**Contributors Assumed (#):** *[Choose one of the following]*	**Persons Reasonably Expected:** *[free type – must enter text (ie. name or N/A)]*	**Profiles for Comparison:** *[Choose one of the following]*	**Exclusions:** *[Free type – must enter text (ie. name or N/A). If reporting major and/or minor, specify (ie. Major: Name).]*	**Inclusions:** *[Free type – must enter text (ie. name or N/A). If reporting major and/or minor, specify (ie. Major: Name).]*	**Statistic:** *[Free type – must enter text (ie. stat or N/A). If reporting major and/or minor, specify (ie. Major: For XX loci, approximately 1 out of…" Or Major: For XX loci, rarer than 1 out of 100 trillion).]*	**Profiles Uploaded to CODIS:** *[Choose one of the following]*
					None – No interpretable data				Yes – partial major & partial minor
					Single source				Yes – partial major only
					Major only, minor data too complex				Yes – partial minor only

16.3.3 For information in paragraph format

16.3.3.1 Paragraph format will be used when the table cannot completely and/or accurately communicate results to the customer. Examples of when the paragraph format may be necessary include, but are not limited to, CODIS requests, Supplemental requests, and to report the submission or comparisons from reference standards. Often reports will

include both table and paragraph information; however, the results should not be redundant.

16.3.3.2 Right click on the request and select EDIT FINDINGS. Right click on the request related to the results. Select ADD RESULT. Below are autotext statements and their codes. Other statements may be used in order to accurately report results and conclusions.

Circumstance	Autotext Code	Statement
When previous Forensic Biology Reports have been issued under the same CL #	SUPP	Refer to the official MNPD Forensic Biology Report(s) dated _____ for previous results.
When liquid blood is submitted, analyzed, and a portion of the blood is retained in dried form	LBT	A bloodstain card was prepared from a sample of Item(s)….
When liquid blood is submitted, not analyzed, and a portion of the blood is retained in dried form	LBNT	A bloodstain card was prepared from a sample of Item(s)….. No testing was performed on this item.
When hair is collected and repackaged	HAC	Possible hair was collected from Item… and labeled as_____.
When items are returned unopened	UNEXAM	Item(s)… is/are being returned unexamined.
When items are opened, but not chemical tested	UNTEST	No testing was performed on Item(s)…
When items are forwarded for DNA	DNA	Item(s)… is/are being forwarded for DNA analysis.
Samples used as a DNA Reference	REFDNA	A DNA profile/ DNA profiles was/were obtained for comparison from sample(s) of Item(s)…
(+) activity detected after assumptions	PACDNA	An indication/Indications of a possible additional contributor was/were present on the sample(s) of Item(s)…

When no further testing is performed on an item (i.e., unstained micro slides)	NFT	No further testing was performed on Item(s)…
When further testing is an option (not all items are fully tested)	FT	If further testing is desired, please contact the analyst for more information.
When DNA reference standards will potentially provide more information	STDS	If DNA standards in relation to the investigation are obtained, please contact the analyst for more information.
Various spellings of names*	SP	Spellings of names can differ among case submission paperwork, evidence packages, and/or paperwork submitted to the laboratory. This/these is/are the alternative name spelling(s) observed for the individual(s) listed above:
Additional testing to follow	FU	Additional testing will be performed on Items….. The results will be issued in a follow up report.
Samples were stopped after quantitation or Y-screen results indicated on report	Select the box in the Edit Request tab in the LIMS	Deoxyribonucleic Acid (DNA) was isolated and quantitated using the Promega Plexor HY Quantitation System
Evidence was outsourced to a vendor laboratory	OUT	Evidence was outsourced to [Bode Technology/DNA Labs International] on [date]. Biological screening and /or Y-screening and/or DNA analysis will be performed as applicable. You will be notified when casework is complete.
Additional testing will be forthcoming (i.e., outsourced case)	ADDTEST	Additional testing will be performed on the above listed evidence.
Additional testing was performed (i.e., outsourced case)	ADDTESTR	Additional testing was performed on the above listed evidence.
Contact the analyst for more information	CNCT	Please contact the undersigned analyst for more information.

DNA profile will be evaluated for CODIS upload (i.e., outsourced case)	CDSEVAL	The DNA profiles in this report will be evaluated for CODIS upload. If uploaded, notification will be made in a separate report.

16.3.3.3 *Use of spelling statement

16.3.3.3.1 Examples of when the spelling statement is needed. The examples are not intended to be all encompassing.

16.3.3.3.1.1 "Jr" is listed on one form, but not other forms.

16.3.3.3.1.2 Middle name is on one form and no middle name is listed on other forms.

16.3.3.3.1.3 Middle initial is different than middle initial on other forms.

16.3.3.3.1.4 It appears names could be different people or if names are misspelled.

16.3.3.3.2 Examples of when a spelling statement is not needed. The examples are not intended to be all encompassing.

16.3.3.3.2.1 One form has middle initial, and one form has middle name spelled out (and they are consistent)

16.3.3.3.2.2 One form has middle initial, and the other form has no middle initial listed.

16.3.3.4 DNA Statements for Supplemental Request:

16.3.3.4.1 When a request includes DNA reference standards only to be compared to previously submitted evidence previously submitted, the results of the comparison will need to be reported in paragraph form since no evidence is associated with the request to allow for drop-down in the table.

Circumstance	**Autotext Code**	**Statement**
Complete Single Source evidence profile identical to DNA Reference profile	SS	The (partial) (foreign/major/minor) DNA profile(s) from Item(s)… match(es)/is(are) consistent with the DNA profile obtained from the sample Item…
Unresolvable mixture	MIX+	_____ is included as a possible contributor to the (partial/foreign) mixed DNA profile(s) from Item(s)…
Calculated statistic below 100 trillion	STAT	For (# used in statistical analysis) loci, the frequency of occurrence of the (interpretable, mixed, major, minor) DNA profile obtained from the sample(s) of

		Item(s)… for unrelated individuals is approximately 1 out of _____.
Calculated statistic above 100 trillion	STATEX	STATEX: For (# used in statistical analysis) loci, the frequency of occurrence of the (interpretable, foreign, mixed, major, minor) DNA profile obtained from the sample(s) of Item(s)… for unrelated individuals is rarer than 1 out of 100 trillion.
DNA Reference profile excluded from the evidence data	XDNA	_____ is excluded as (the source of the foreign/major/minor DNA profile/ a possible contributor to the mixed DNA profile) obtained from the sample(s) of Item(s)…

16.3.3.5 Auto text statements for Supplement Serology reports in paragraph form

Circumstance	Autotext Code	Statement
When ALS testing is performed	ALS	A test/Tests for the detection of biological fluids was performed on the sample(s) of this/these Item(s) using the alternate light source (ALS). The result was….
When KM and HemaTrace or HemaTrace only is performed	KM/RSID	(A) Test(s) for the detection of blood was/were performed using Kastle-Meyer (KM) (and RSID) on the sample(s) of this/these Item(s). The results was/were (test) (result).
When KM only is performed	KM	A presumptive test gave/did not give chemical indications of blood on the sample(s) of this/these item(s).
When KM is inconclusive	KM?	A presumptive test for blood was inconclusive on the sample(s) of this/these Item(s) due to the results being outside the laboratory's validated parameters.
When AP only is performed	AP	A presumptive test/Presumptive tests gave/did not give chemical indications of semen on the sample(s) of this/these Item(s).
When AP and p30 or p30 only is performed	PRS	A Test(s) for the detection of seminal fluid was/were performed using (test(s)) on

		the sample(s) of this/these Item(s). The result(s) was/were (test) (result).
When AP and sperm search are performed		Tests for detection of seminal fluid and sperm were performed using an acid phosphate test (AP) and microscopic examination on Item (X). The results were AP (result) and sperm (result).
When sperm search only is performed	Micro	A test/Tests for the detection of sperm was performed using microscopic examination o the sample(s) of this/these Item(s). The result was sperm (result).
When AP, p30, and sperm search are performed	TRS	Tests for the detection of seminal fluid and sperm were performed using (an acid phosphatase (AP) test, ABA card p30 test), and microscopic examination on the sample(s) of this/these Item(s). The results were AP (result), p30 (result), and sperm (result). Manually add the following to the end of the above statement: Acid phosphatase is an enzyme found in generally high levels in seminal fluid and in relatively lower levels in several other substances. The antigen p30 is a protein found in generally high levels in seminal fluid and in relatively lower levels in some other bodily fluids.

16.3.3.5.1 **Note**: Reports are not limited to the examples listed above. There might be circumstances to word the results differently; however, the results should not be redundant.

16.3.3.6 CODIS Statements:

16.3.3.6.1 If applicable, the following autotext statements related to CODIS upload shall be added to appropriate items following the same procedure described above. Additional statements may be used in order to accurately report any CODIS activity.

Circumstance	Autotext Code	Statement
Keyboard Search	CDSKB-	The (partial) (unknown/foreign/major/minor/mixed) DNA profile developed from the sample(s) of Item(s)…was/were searched in the

		CODIS database and no/a match was detected as of this report date.
Uploaded profiles	DBSEARCH	(An) STR profile(s) from the following item(s) has/have been entered into the Combined DNA Index System (CODIS) in accordance with local, state, and national regulations where regular searches will be performed: <item descriptions>. Notifications will be issued if there is a hit in the database or if a profile is removed from CODIS at any time in the future.
Removal due to elimination reference standard	CDSELIM	The (partial) (unknown/foreign/major/minor/mixed) DNA profile(s) from the sample(s) of Item(s)… has been removed from the CODIS database after comparison to (elimination reference standard).
Profile removed due to eligibility requirements	CDSREMOVE	The (partial) (unknown/foreign/major/minor/mixed) DNA profile(s) from the sample(s) of Item(s)… has been removed from the CODIS database. The profile does not meet the current CODIS eligibility requirements.
Case to Case Hit	CASEHIT	During a search of the CODIS database, a (high/moderate) stringency association was made between the (partial) (unknown/foreign/major/minor/mixed) DNA profile(s) obtained from the sample(s) of Item(s)… and (laboratory agency) Lab #_____ (agency, agency case #, officer). For additional information, please contact the undersigned analyst.
Evidence to DNA Reference Hit	REFHIT	During a search of the CODIS database, a (high/moderate) stringency association was made between the (partial) (unknown/foreign/major/minor/mixed) DNA profile(s) obtained from the sample(s) of Item(s)… and (laboratory agency) Lab #_____. The information being provided is an investigative lead only. In order to confirm this, a blood or buccal sample from (Name) (DOB) must be submitted for DNA testing.

With all Hit Reports	CDSRMS	Additional information may be found in RMS.

16.3.3.6.2 Click APPLY to assign these findings.

16.3.3.7 EDIT REQUEST Statements:

16.3.3.7.1 Additional information is necessary prior to submitting a report for technical review. Right click the request and select EDIT REQUEST. Click on the button that looks like … (beside "Complexity"). Complete all applicable data extension fields. The "Examination Start Date" shall reflect the date on which the laboratory process began (i.e., date of first inventory sheet or extraction sheet, if samples were previously cut and documented in screening report). For the "Disposition," one of the following two statements is commonly used:

Circumstance	**Autotext Code**	**Statement**
Disposition for Casework and CODIS Reports	DISP	The remainder of the evidence is being returned/forwarded/transferred to_____.
When a high stringency association from the DNA database is reported	HSTRING	High Stringency Association – Exact match between the compared profiles
When a moderate stringency association from the DNA database is reported	MODSTRING	Moderate Stringency Association - 1) Involves the comparison of mixture(s) or 2) the data present matches between the profiles being compared, but information may be missing in at least one of the profiles

16.3.3.8 In addition, check all applicable optional statements that apply to the corresponding report:

16.3.3.8.1 PCR was performed

16.3.3.8.1.1"…STR statement on final report"

16.3.3.8.2 Statistical calculations were performed

16.3.3.8.2.1"…DAT1 statement on final report"

16.3.3.8.3 Samples were stopped after quantitation

16.3.3.8.3.1"…Quant statement on final report"

16.3.3.8.4 Report is an amended report

16.3.3.8.4.1"…amended final report"

16.3.3.8.5 CODIS Associations are reported

16.3.3.8.5.1"…associations on final report"

16.3.3.9 If the report is amended, enter the original report date. Also, state what changes were made to the report in the "Additional Amend Statement – Optional" data extension field.

16.3.3.10 Other applicable metrics should be included under "Number of Tests Completed."

16.3.3.11 Once all applicable fields have been completed, click APPLY.

16.3.3.12 Right click on the request and select PRINT FINAL REPORT. Select the report to be printed to Screen.

16.3.3.13 Review the report to ensure fields which were auto-populated from the 282 form and the autotext statements are correct. If report is correct, close the screen containing the draft and right click on the request. Select SET MILESTONE then DRAFT COMPLETE. Select YES. In the Sign Report box, mark the circle next to SIGN, select ANALYST in the drop-down menu, and click in the field below. The analyst must scan or type individual barcode and type the PIN number. Click OK.

16.4 Limitations

16.4.1 A final report will not be released to a client until a successful technical and administrative review on the case has been completed.

16.5 References

16.5.1 MNPD-CL Forensic Biology Technical Procedures Manual

17. Case Review

17.1 Scope

17.1.1 To describe the process for performing the technical review on completed batch work and performing technical and administrative reviews on completed Forensic Biology casefiles.

17.2 Equipment/Materials/Reagents

17.2.1 Completed batch work

17.2.2 Forensic Biology Processing Batch Form and ReviewForm , if applicable

17.2.3 Completed electronic casefile

17.2.4 Forensic Biology Case Review Form or Forensic Biology Serology Review Form

17.2.5 LIMS and Adobe software

17.2.6 GeneMapper® ID-X Software v1.6

17.2.7 MNPD-CL network

17.2.8 QMS

17.3 Procedure

17.3.1 Completed batch work

17.3.1.1 Open the DNA Batch Workbook located on the MNPD-CL network.

17.3.1.2 Open the review form associated with the batch. The analyst review should be complete prior to submitting the batch of cases for technical review.

17.3.1.3 Elements listed in the Processing Batch Form and Review Form should be checked during the technical review of the batch. To notate suggestions, need for corrections, or requests for clarification in the DNA Batch Workbook or witness sheet(s), post comments on the PDF using the Comments.

17.3.1.4 Use of QMS, the MNPD-CL network, Plexor Software and GeneMapper ID-X should be utilized for the review.

17.3.1.5 The analyst will then be notified that a batch review has been conducted. The analyst should ensure that the comments are not deleted as responses are made to the reviewer's comments.

17.3.1.6 Once corrected, the analyst will notify the reviewer that corrections are complete. Once all the elements of the batch review are verified, have the analyst add "Workbook Locked" with date and initials and lock the DNA Batch Workbook PDF. The reviewer will electronically sign the technical reviewer box of the Processing Batch Form and Review Form.

17.3.2 Completed electronic case file

17.3.2.1 Open the electronic case file located on the MNPD-CL network.

17.3.2.2 Open the review form associated with the case file. The analyst review should be complete prior to submitting the case file for technical review.

17.3.2.3 Elements listed in the technical review portion of the Forensic Biology Case Review Form, or the Forensic Biology Serology Review Form should be checked during the technical review. To notate suggestions, need for corrections, or requests for clarification in the case file, post comments on the PDFs using Adobe.

17.3.2.4 Review the case report and chain of custody in the LIMS software. If any comments or suggestions are needed for the case report, it must be printed from the LIMS to PDF on the MNPD-CL network.

17.3.2.5 If a discrepancy or needed correction is identified, the technical reviewer will select the option to 'Reject Findings' in the LIMS and describe the discrepancy or needed correction citing the reference to policy (if applicable) in the 'Reviewer Notes' for the request. Right click on the request to select Reject Findings.

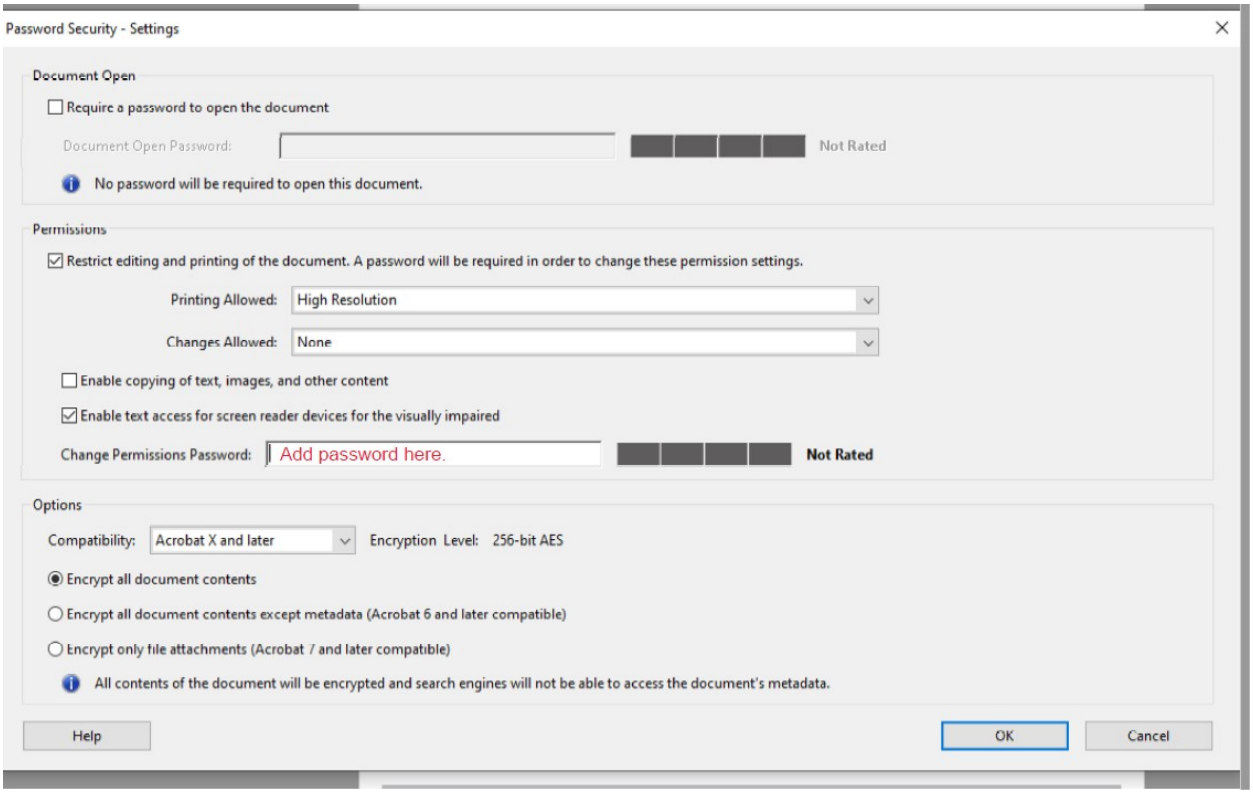
17.3.2.5.1 Additionally, the following information should be listed in the comment section: initials of individual performing the review, date of rejection, type of review (Tech or Admin review), and type of errors prompting the rejection (technical error, administrative error, or technical and administrative error).

17.3.2.6 The analyst will also be notified via Microsoft Planner about any corrections needed or if the case is ready to prepare for administrative review. The analyst should ensure that the comments are not deleted as responses are made to the reviewer's comments.

17.3.2.7 Once corrected, the analyst will notify the reviewer that corrections are complete via Microsoft Planner. Once all the elements of the technical review are verified, electronically sign the technical reviewer box of the Forensic Biology Case Review Form and set the Technical Review milestone in the LIMS.

17.3.2.7.1 To set the Technical Review milestone, open the main LIMS software and open the appropriate case file. Click on the Request tab and right click the appropriate request. Select Set Milestone, and Technical Review. Select Sign, scan the reviewer's personal barcode or type the user name in the barcode area and type in PIN.

17.3.2.8 The case file should now be prepared for administrative review by adding a coversheet and securing the case file. Secure the case file by clicking on the Protect tool in Adobe. Click on Advanced Options and then Encrypt with Password. Click Yes on the next dialog box. Fill in the settings as listed below.



17.3.2.9 The case file is now ready for an administrative review (alternatively, portions of the administrative review may be performed in conjunction with the technical review).

17.3.2.10 Elements listed in the review form administrative review section should be checked during the administrative review. Ensure at least one draft report is present in the case file folder.

17.3.2.11 If a discrepancy or needed correction is identified, the administrative reviewer will select the option to 'Reject Findings' in the LIMS and describe the discrepancy or needed correction citing the reference to policy (if applicable) in the 'Reviewer Notes' for the request. Right click on the request to select Reject Findings.

17.3.2.11.1 Additionally, the following information should be listed in the comment section: initials of individual performing the review, date of rejection, type of review (Tech or Admin review), and type of errors prompting the rejection (technical error, administrative error, or technical and administrative error).

17.3.2.12 The analyst will also be notified via Microsoft Planner about any corrections needed or if the case has been reviewed and released.

17.3.2.13 Once all the elements of the administrative review have been verified, electronically sign the administrative reviewer check box of the Forensic Biology Case Review Form. Finally, set the Administrative Review milestone in the LIMS.

17.3.2.13.1 To set the Administrative Review milestone, open the main LIMS software and open the appropriate case file.

17.3.2.13.1.1 Click on the Request tab and right click the appropriate request.

17.3.2.13.1.2 Select Set Milestone, and Administrative Review.

17.3.2.13.1.3 Select Sign, scan the reviewer's personal barcode or type the username in the barcode area and type in PIN.

17.3.2.13.1.4 Verify the analyst's signature has been added to the report in the LIMS by opening the released report.

17.3.2.14 Rename the case file folder to add the assignment that is listed on the Form 282 (ex. CL-21-003325 Casefile MDM - CID). Cut and paste this folder to the following location in the Completed folder depending on the month and year that the case was released: L:\Crime Lab Network Drive\Forensic Biology\Forensic Biology Unit\Electronic Casefiles\Completed.

17.3.2.14.1 The administrative reviewer will then zip the case file folder (Right click on folder -> Send to -> Compressed (zipped) folder). Move the zip file into the original folder.

17.3.2.14.2 Create a Sub Folder in the LIMS software under Case Attachments named DNA Request or Serology Request followed by the Request number (ex. DNA Request 0001). Upload the zip file into this Sub Folder after the case has been released. Leave the zip file in the original folder.

17.4 References

17.4.1 Forensic Biology Case Review Form

17.4.2 Forensic Serology Case Review Form

17.4.3 Processing Batch Form and Review Form