Technical Procedure for Screen for DNA Analysis

1.0 Purpose – This technical procedure shall be followed for the examination of screen for DNA hair evidence.

2.0 Scope – This procedure applies to all hair cases in the Trace Evidence Section which require the examination of hair evidence for roots that may be suitable for DNA analysis.

3.0 Definitions – N/A

4.0 Equipment, Materials, and Reagents

4.1 Equipment

- Stereomicroscope
- Compound or Polarized Light Microscope
- Comparison microscope

4.2 Materials

- Glass microscope slides and cover slips
- Forceps
- Scalpel and blades
- Scribe
- Microcentrifuge tubes
- Well plates
- Fingerprint lifting tape
- Pasteur Pipettes
- Probes

4.3 Reagents

- Xylene
- Xylene substitute
- Methanol
- Absolute Ethanol (anhydrous) 200 proof
- Deionized water
- 10 % bleach solution
- Modified Harris Hematoxylin

5.0 Procedure

5.1 Analytical Approach

5.1.1 Review the request for analysis.

5.1.2 Open the evidence container and describe the evidence present.

5.1.3 Process the item to remove any hair evidence adhering to the item following the Trace Evidence Section Technical Procedure for the Collection and Preservation of Evidence.

5.1.3.1 If processing evidence in a Sexual Assault Evidence Collection Kit (SAECK), see the SAK Processing Guidance Form.

5.1.4 If no questioned hair is present, the examination shall conclude.

5.1.5 If questioned hair evidence is present, use a stereomicroscope to screen for human hairs with roots that may be suitable for DNA analysis at this laboratory. Temporarily mounting (see **Sampling and Sample Selection** and **Sample Preparation**) the hair may be necessary to assist in determining root suitability.

5.1.5.1 Determine if the hair is of human or animal origin.

5.1.5.1.1 A determination regarding ancestral group or somatic origin classification of any human hairs will not be performed unless case circumstances dictate otherwise or specifically requested by the investigating agency. See the Trace Evidence Section Technical Procedure for Microscopic Hair Analysis for guidance in these cases.

5.1.5.1.2 A force determination may be performed on any human hairs.

5.1.5.1.2.1 If the hair has a root, the growth phase and structure of the hair root is examined to determine if it could have been forcibly removed.

5.1.5.1.2.2 If there is no root present, a force determination cannot be performed.

5.1.5.2 View the root area to assess DNA suitability.

5.1.5.2.1 If human hairs with roots that may be suitable for DNA analysis are present, the examination continues (see **Submitting Hairs for DNA Analysis**).

5.1.5.2.2 If no human hairs are suitable for DNA analysis, the examination is complete. Ensure any hairs are secure (e.g., placed on a piece of tape, placed on a post-it note) prior to returning the evidence to the packaging.

5.1.6 At the completion of the examination, the Forensic Scientist shall issue a report stating his or her findings using the **Guidelines for Screen for DNA Examination Result Statements** as a guide.

5.2 Sample Preparation

5.2.1 If questioned hairs are being removed from tapings, carefully remove the hair from the taping using a solvent such as Xylene or Methanol.

5.2.2 Place the hair on a clean microscope slide and apply a temporary mounting medium, such as water or xylene, until the hair is completely covered. Place a glass cover slip on top of the hair and mounting medium.

5.3 Submitting Hairs for DNA Analysis

5.3.1 DNA analysis can be conducted on hairs in the anagen or catagen growth phase or on telogen roots that contain greater than 10 nuclei. If no roots are suitable for DNA analysis, no further analysis can be conducted by this Laboratory. Mitochondrial DNA analysis may be recommended.

5.3.1.1 The number of nuclei present in a telogen root is determined by staining with Modified Harris Hematoxylin (See **Hair Roots in the Telogen Growth Phase**).

5.3.1.2 Telogen hair roots are assessed by the Forensic Scientist to determine which roots need to be stained. When a root does not need to be stained (i.e., no follicular tissue present), no further analysis is needed.

5.3.2 Based on the length of the hair, determine whether the entire hair or only the hair root will need to be sent for DNA analysis.

5.3.3 Sterilize all tools prior to use.

5.3.4 If the hair has been temporarily mounted in water or xylene, remove the hair from the slide.

5.3.5 If the hair has been mounted in a medium such as Permount or Cytoseal, carefully break the cover slip around the questioned hair and/or hair root using a scribe. A drop of xylene on the exposed area will dissolve the mounting media and allow the hair or hair root to be removed.

5.3.5.1 Upon removal from the slide, rinse the hair and/or hair root in xylene to remove any adhering mounting media.

5.3.6 Continue with the analysis based on the root growth phase of the hair.

5.3.7 Hair Roots in the Anagen or Catagen Growth Phase

5.3.7.1 Remove the root of the hair using a sterile blade unless the hair is of insufficient length to have the root area removed.

5.3.7.2 Thoroughly rinse the hair or hair root in deionized water, followed by absolute ethanol.

5.3.7.3 Place the hair or hair root into a labeled microcentrifuge tube and assign a root sub-item number.

5.3.7.4 Unless the hair has been submitted in its entirety, label any remaining portion of the hair with the corresponding root sub-item number.

5.3.7.4.1 Any portion of the hair remaining on the glass microscope slide shall be labeled with the corresponding root sub-item number on the slide.

5.3.7.4.2 Any remaining portion of the hair that is no longer preserved on a glass microscope slide shall be secured (e.g., placed on a piece of tape, placed on a post-it note) and labeled with the corresponding root sub-item number.

5.3.8 Hair Roots in the Telogen Growth Phase

5.3.8.1 The hair root screening procedure described below uses Modified Harris Hematoxylin to stain any nuclei present in the root area of a hair in the telogen growth phase. The stained nuclei are then counted to determine the root's suitability for DNA analysis.

5.3.8.1.1 Soak the root end of the hair in absolute ethanol for 30 minutes.

5.3.8.1.2 Soak the root end of the hair in Modified Harris Hematoxylin for 3 minutes.

5.3.8.1.3 Rinse the root end of the hair with deionized water followed by absolute ethanol.

5.3.8.1.4 Place the hair on a microscope slide and temporarily mount in xylene or xylene substitute.

5.3.8.1.5 View the stained root with a transmitted light microscope and examine for the presence of nuclei. The nuclei are dark red or purple in color and usually oval in shape. Count the visible nuclei.

5.3.8.1.5.1 The root may also be viewed using fluorescence microscopy which will cause the nuclei to fluoresce different colors depending on the filter used.

5.3.8.1.6 If more than 10 nuclei are present the entire hair may be sent for DNA analysis or the root may be removed using a sterile blade and sent for DNA analysis.

5.3.8.1.6.1 Place the hair or hair root into a labeled microcentrifuge tube and assign a root sub-item number.

5.3.8.1.6.2 Secure (e.g., placed on a piece of tape, placed on a post-it note) any remaining portion of the hair and label with the corresponding root sub-item number.

5.3.8.1.7 If less than or equal to 10 nuclei are present, the root will not be removed and the hair may be recommended for mitochondrial DNA analysis.

5.3.8.1.7.1 Ensure the hair is properly secured (e.g., placed on a piece of tape, placed on a post-it note) prior to returning the evidence to the packaging.

5.3.9 Place any sub-items created for DNA analysis into secure packaging, label as a Container in FA, and transfer to the Forensic Biology Section.

5.4 Guidelines for Screen for DNA Examination Result Statements

5.4.1 A methodology statement shall be added to all reports.

5.4.1.1 Example: The following methodologies were used in the examination of this case: visual examination, hair root staining, and microscopy.

5.4.2 The wording of the results shall accurately describe the evidence at hand.

5.4.3 The report shall address all questioned hairs present in a case, whether analyzed or not.

5.4.4 Screen for DNA

5.4.4.1 If there are hairs with roots suitable for DNA analysis:

5.4.4.1.1 Example: Examination of Item A revealed the presence of several hairs with roots that may be suitable for DNA analysis.

5.4.4.2 If the roots of the hairs were removed:

5.4.4.2.1 Example: The roots of these hairs were removed, assigned Item(s) #___ and sent for DNA analysis.

5.4.4.3 If the hairs were retained but roots were not removed:

5.4.4.3.1 Example: Examination of Item A revealed the presence of several hairs. _____ of these hairs had roots that may be suitable for DNA analysis. These hairs have been assigned Item(s) # _____ and will be retained in the laboratory. The remaining hairs were not suitable for DNA analysis. No further examination was performed.

5.4.4.4 Retained hairs sent back to the Trace Evidence Section for root removal:

5.4.4.4.1 Example: Item # ____ was previously analyzed by _____. The results of that analysis can be found in the laboratory report dated _____. Item # _____ was retained in the laboratory in the event the roots needed to be removed for DNA analysis. The roots from these hairs were removed, assigned Item(s) # ____ and sent for DNA analysis.

5.4.4.5 If no evidence is suitable for DNA analysis:

5.4.4.5.1 Example: Examination of Item A revealed the presence of several hairs. No hairs suitable for DNA analysis were noted. No further analysis was performed on this item.

5.4.4.6 If the hair was sent for DNA analysis in its entirety:

5.4.4.6.1 Example: This hair was assigned Item A-1 and submitted in its entirety for DNA analysis.

5.4.5. No Analysis

5.4.5.1. No analysis is performed due to the outcome of DNA analysis

5.4.5.1.1. DNA results correlate two items of evidence (e.g., suspect's DNA profile is identified on the victim's vaginal swabs).

5.4.5.1.1.1 Example: Based on the results of DNA analysis, the above listed evidence is being returned without analysis. If you have any questions, please contact the Forensic Scientist who issued this report.

5.4.5.1.2 An unknown DNA profile was developed on an item of evidence (e.g., vaginal swabs).

5.4.5.1.2.1 Example: Due to the fact that there is an unknown DNA profile noted in the Forensic Biology report dated *mm/dd/yy* by *analyst*, the above listed evidence is being returned without examination at this time. If you have any questions, please contact the Forensic Scientist that issued this report.

5.4.5.2 No questioned hair evidence present

5.4.5.2.1 Example: Because no questioned hair evidence was submitted for analysis, the above listed known standards are being returned without examination. If you have any questions, please contact the Forensic Scientist who issued this report.

5.4.5.2.2 Example: Examination of Item A did not reveal the presence of any hairs.

5.4.5.3 Improper Collection of Hair Evidence

5.4.5.3.1 Example: Item A was improperly collected/packaged and will be returned without examination.

5.4.5.4 Common Environment

5.4.5.4.1 Example: Because it cannot be determined when or how a hair was deposited on an item from an environment common to both the victim and suspect, a hair analysis cannot be performed on Item A.

5.4.6 Qualifying Statements

5.4.6.1 Qualifying statements shall be included in the formal report if their inclusion further explains the conclusion or provides necessary information to the reader regarding the interpretation of the conclusion.

5.4.6.2 Qualifying Statement Regarding Force Determination

5.4.6.2.1 When determining if a hair has been forcibly removed, it is not possible for the Forensic Scientist to determine how, when, or why the hair was forcibly removed. A qualifying statement shall be added to communicate this.

5.4.6.3 Qualifying Statement Regarding Suitability for Hair Comparison

5.4.6.3.1 A statement regarding the option for microscopic hair comparison shall be included on the report when the hair analyst has exhausted the DNA capabilities of the North Carolina State Crime Laboratory and hairs remain that may be suitable for a microscopic hair comparison.

5.4.6.4 Qualifying Statement Regarding DNA Analysis

5.4.6.4.1 A statement regarding the option for DNA testing shall be included on the report when the hair analyst has exhausted the examination capabilities of the North Carolina State Crime Laboratory and questioned hairs remain that may be suitable for DNA analysis not performed at this laboratory.

5.5 Standards and Controls

5.5.1 Quality assurance checks shall be performed upon receipt of a new lot of Modified Harris Hematoxylin.

5.5.1.1 The efficiency and performance of a new lot of hematoxylin shall be verified prior to use by staining a hair root with adhering tissue and noting where a color change occurred. The results will be recorded in Forensic Advantage Resource Manager.

5.6 Calibration – N/A

5.7 Maintenance – No maintenance is required in this procedure. However, the procedure does utilize instruments that require maintenance. See the individual technical procedures for the operations of those instruments.

5.8 Sampling and Sample Selection

5.8.1 No sampling is performed. When sample selection occurs, such as determining whether to stain a telogen hair with no root tissue adhered, it shall be based on the Forensic Scientist's training and experience.

5.8.2 Sample Selection Guidelines

5.8.2.1 If a number of questioned hairs are submitted from the same location and are believed to have been deposited at the same time during the same event (e.g., a clump of hairs, dreadlock), they may be treated as a group.

5.8.2.2 If a large quantity of hairs is present in a clump, a number of the questioned hairs shall be selected by the Forensic Scientist as representative of the entire questioned sample.

5.8.3 Situations in which examinations may be discontinued are as follows:

5.8.3.1 Based on the results of DNA analysis, the hair evidence may be returned unanalyzed.

5.8.3.2 If the DNA report states that an unknown profile has been found in an item of evidence that would provide the same information as the hair analysis, the hair evidence may be returned pending the identification of the unknown profile (e.g., unknown male profile on the victim's vaginal swabs would mean the victim's pubic hair combings could be returned unanalyzed until the unknown profile is identified).

5.8.3.3 If questioned items have been improperly collected, the evidence may be returned to the agency unanalyzed.

5.8.3.4 If it is known that the parties involved in the case share a common environment, the evidence may be returned to the agency unanalyzed.

5.8.3.5 For SAECK cases, see the SAK Processing Guidance Form.

5.9 Calculations – N/A

5.10 Uncertainty of Measurement – N/A

6.0 Limitations

6.1 When a human hair does not have a root suitable for the DNA testing options offered at this Laboratory, it will be recommended that samples be outsourced for additional DNA testing when the results of a hair examination may establish an association (e.g., victim to suspect, victim to scene).

7.0 Safety

7.1 Items may have blood or other body fluids present. Use protective equipment when dealing with items that may contain biohazard material. Refer to Laboratory Safety Manual: Bloodborne Pathogen Compliance Program.

7.2 Care shall be exercised when using Modified Harris Hematoxylin and solvents such as xylene and xylene substitute. Consult Safety Data Sheets for information on safe use for reagents listed in this procedure and refer to the Laboratory Safety Manual- Chemical Hygiene Plan and Hazardous Communication Program.

7.3 Glass pipettes, razor blades, and probes are sharp and can be dangerous.

8.0 References

8.1 ASTM Guidelines

SWGMAT. "Forensic Human Hair Examination Guidelines." *Forensic Science Communications* 7.2 (2005).

8.2 Books

DeForest, P.R., R.E. Gaensslen and H.C. Lee. *Forensic Science: An Introduction to Criminalistics*. New York: McGraw-Hill, 1983.

F.B.I. *Proceedings of the International Symposium on Forensic Hair Comparisons*. Washington, D.C.: The Laboratory Division, 1985.

Gaudette, B.D. *The Forensic Aspects of Hair Examination*. RCMP, Central Forensic Laboratory, 1988.

Robertson, J., ed. *Forensic Examination of Hair*. London: Taylon & Francis, 1999.

Saferstein, R., ed. *Forensic Science Handbook*. Volume I. Englewood Cliffs, NJ: Prentice Hall, 1983.

8.3 Journal Articles

Linch, C., S. Smith and J. Prahlow. "Evaluation of the Human Hair Root for DNA Typing Subsequent to Microscopic Comparison." *Journal of Forensic Sciences* 43.2 (1998): 305-314.

Melton, T., et al. "Forensic Mitochondrial DNA Analysis of 691 Casework Hairs." *Journal of Forensic Sciences* 50.1 (2005):73-80.

Houck, M. and B. Budowle. "Correlation of Microscopic and Mitochondrial DNA Hair Comparisons." *Journal of Forensic Sciences* 47.5 (2002): 964-967.

Bourguignon, L., et al. "A Fluorescent Microscopy-Screening Test for Efficient STR-Typing of Telogen Hair Roots." *Forensic Science International: Genetics* 3 (2008): 27-31.

Brooks, E.M., et. al. "Nuclear Staining of Telogen Hair Roots Contributes to Successful Forensic nDNA Analysis." *Australian Journal of Forensic Sciences* 42.2 (2010): 115-122.

Edson, J., et. al. "A Quantitative Assessment of a Reliable Screening Technique for the STR Analysis of Telogen Hair Roots." *Forensic Science International: Genetics* 7 (2013): 180-188.

9.0 Records

Laboratory Safety Manual- Chemical Hygiene Plan and Hazardous Communication Program.

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
06/21/2023	2	Added Trace Evidence Section before any procedural references throughout document Updated unknown hair to questioned hair throughout Clarified absolute ethanol throughout 4.3 – Added Methanol Added 5.1.3.1 5.1.5 – Added "at this laboratory" 5.1.5.1 – Clarified only human versus animal determination Added 5.1.5.1.1 and 5.1.5.1.2 Added 5.1.5.2 5.1.5.2 became 5.1.5.2.1 – Clarified human hairs 5.1.5.3 became 5.1.5.2.2 – Clarified human hairs

		Added 5.2.1 Added 5.3.1.1 and 5.3.1.2 Clarified 5.3.2 Removed 5.3.2.1 and 5.3.2.2 Clarified 5.3.7.3 5.3.7.4 and 5.3.8.1.6 – Removed due to insufficient length Clarified 5.3.8.1.6.1 Added 5.3.9 Clarified 5.4.2.4 Removed 5.4.4.2 and related sub-headers 5.4.4.3.1 – Removed Some and Added _____ 5.4.6.1 and 5.4.6.4.1 – Removed reference to the Trace Evidence Section Technical Procedure for Microscopic Hair Analysis procedures Added 5.4.6.2, 5.4.6.3, and related sub-headers Added 5.5.1 and 5.5.1.1 Clarified 5.8.2.2 Removed 5.8.2.3 Removed 5.8.3.1 5.8.3.2 – Removed of the appropriate gender Added 5.8.3.5 6.1 - Clarified additional DNA testing 7.2 – Added Modified Harris Hematoxylin; removed reference to appendix 1 9.0 – Removed redundant reference Removed appendix 1