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
- 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.

5.1.4 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. 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 The macroscopic and/or microscopic characteristics of the hair may be evaluated according to the Technical Procedure for Microscopic Hair Analysis to aid in sample selection.

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

5.1.5.3 If no 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, etc.) 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 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.

5.2.2 The hairs shall be retrieved, secured (if necessary) and placed into the original packaging after the examination is complete.

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.

5.3.2 Determine whether the entire hair or only the hair root will need to be sent for DNA analysis.

5.3.2.1 If the hair is of sufficient length, only the root area of the hair shall be removed and sent for DNA analysis.

5.3.2.2 If the hair is of insufficient length, the entire hair shall 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 100 % ethanol.

5.3.7.3 Place the hair or hair root into a labeled microcentrifuge tube. Place the tube into a labeled manila envelope.

5.3.7.4 Unless the hair has been submitted in its entirety (due to insufficient length), 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, etc.) 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 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 nuclei may also be viewed using fluorescence microscopy. They will fluoresce different colors depending upon the filter used.

5.3.8.1.6 If more than 10 nuclei are present the entire hair (if insufficient length) 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. Place the tube into a labeled manila envelope.

5.3.8.1.6.2 Secure (e.g. placed on a piece of tape, placed on a post-it note, etc.) 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, etc.) prior to returning the evidence to the packaging.

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 unknown 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 some hairs are being included/excluded on the basis of race (i.e., when suspect and victim are of different races):

5.4.4.2.1 Example: Examination of Item A revealed the presence of several hairs with macroscopic/microscopic Negroid characteristics that may be suitable for DNA analysis.

5.4.4.2.2 Example: Item A was examined for the presence of hairs with macroscopic/microscopic Caucasian characteristics. No hairs of this type were noted.

5.4.4.3 If the roots of the hairs were removed:

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

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

5.4.4.4.1 Example: Examination of Item A revealed the presence of several hairs. Some 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.

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

5.4.4.5.1 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.

5.4.4.6 If no evidence is suitable for DNA analysis:

5.4.4.6.1 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.

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

5.4.4.7.1 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. Examples of qualifying statements can be found in the Technical Procedure for Microscopic Hair Analysis.

5.4.7 Qualifying Statement Regarding Mitochondrial DNA Analysis

5.4.7.1 A statement regarding the option of mitochondrial 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 mitochondrial DNA analysis. Examples of qualifying statements can be found in the Technical Procedure for Microscopic Hair Analysis.

5.5. Standards and Controls – N/A

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 unknown 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, etc.), 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 unknown hairs shall be selected by the Forensic Scientist as representative of the entire unknown sample. The selection shall be based primarily on characteristics such as length, coarseness, and color as observed by the Forensic Scientist.

5.8.2.3 Hairs found to be suitable for DNA analysis may be excluded on the basis of race if the race of the subject is known (i.e., suitable root on a Caucasian hair from a Caucasian victim's clothing does not require submission for DNA analysis if the subject is Negroid).

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

5.8.3.1 Pubic hair combings collected more than 48 hours after the incident occurred shall not be analyzed.

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

5.8.3.3 If the DNA report states that an unknown profile of the appropriate gender 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).

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

5.9. Calculations – N/A

5.10 Uncertainty of Measurement – N/A

6.0 Limitations

6.1 This Laboratory does not perform mitochondrial DNA testing; therefore, it will be recommended that samples be outsourced for mitochondrial DNA testing when the results of a hair examination may establish an association between the victim and suspect.

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 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. See **Appendix 1** for chemical hygiene and safety precautions.

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

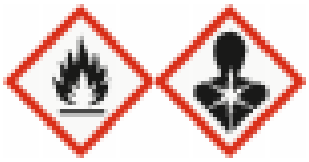
Laboratory Safety Manual- Chemical Hygiene Plan and Hazardous Communication Program.

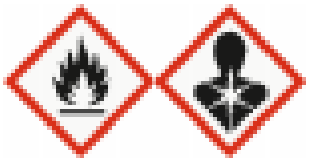
Laboratory Safety Manual- Chemical Hygiene Plan and Hazardous Communication Program.

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
09/21/2020	1	Original Document, Created from Technical Procedure for Hair Analysis

Appendix 1: Chemical Hygiene and Safety Precautions

Xylenes **DANGER: HIGH RISK SUBSTANCE***	
	**HEALTH 2** **FLAMMABILITY 3** **REACTIVITY 0**
Detection of Release	Clear, colorless liquid with a sweet odor.
Signs/Symptoms of Exposure	Breathing difficulties. Respiratory irritation. Skin irritation.
PEL	OSHA PEL - 100 ppm
Associated Hazards	Highly flammable liquid and vapor. **May be fatal if swallowed and enters airways.** Causes skin irritation. May cause respiratory irritation. **May cause damage to organs (Central nervous system, Liver, Kidney) through prolonged or repeated exposure if inhaled.** Toxic to aquatic life.
Controls	Use under fume hood. Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product. Use eye protection. Wear lab coat. Handle with gloves (Nitrile breakthrough time = 35 minutes)
Safe handling, storage, disposal	Handling: Avoid contact with skin and eyes. Avoid inhalation of vapor or mist. Keep away from sources of ignition. Take measures to prevent the build-up of electrostatic charge. Storage: Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage. Dispose in Hazardous Chemical Waste.
Emergency Procedures	**Eye Contact:** Flush eyes with water as a precaution. **Inhalation Exposure:** If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician. **Ingestion:** Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician. **Skin Contact:** Wash off with soap and plenty of water. Consult a physician. **Spills:** Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Evacuate personnel to safe areas. Beware of vapors accumulating to form explosive concentrations. Vapors can accumulate in low areas. Small contained spill: wearing appropriate PPE, collect with non-combustible absorbent material, (e.g. sand, earth, diatomaceous earth, vermiculite) and place in container. Dispose in Hazardous Chemical Waste. Large spills: Evacuate area and call 911 (Haz Mat).

<table>
<tr><th colspan="2">Xylene Substitute
DANGER: HIGH RISK SUBSTANCE*</th></tr>
<tr><td>  </td><td>HEALTH 2
FLAMMABILITY 3
REACTIVITY 0</td></tr>
<tr><td>Detection of Release</td><td>Colorless liquid; Odorless</td></tr>
<tr><td>Signs/Symptoms of Exposure</td><td>Breathing difficulties. Skin irritation.</td></tr>
<tr><td>PEL</td><td>OSHA TWA 500 ppm</td></tr>
<tr><td>Associated Hazards</td><td>Highly flammable liquid and vapor. May be fatal if swallowed and enters airways.</td></tr>
<tr><td>Controls</td><td>Use under fume hood. Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product. Use eye protection. Handle with gloves. Wear lab coat.</td></tr>
<tr><td>Safe handling, storage, disposal</td><td>Handling: Use protective equipment. Do not get in eyes, on skin, or on clothing. Use only under a chemical fume hood. Do not breathe vapors or spray mist. Do not ingest. Keep away from open flames, hot surfaces and sources of ignition. Use only non-sparking tools. Use explosion-proof equipment. Take precautionary measures against static discharges. To avoid ignition of vapors by static electricity discharge, all metal parts of the equipment must be grounded. Storage: Keep containers tightly closed in a dry, cool and well-ventilated place. Keep away from heat and sources of ignition. Dispose in Hazardous Chemical Waste.</td></tr>
<tr><td>Emergency Procedures</td><td><u>Eye Contact</u>: Flush eyes with water as a precaution.
<u>Inhalation Exposure</u>: If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.
<u>Ingestion</u>: Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.
<u>Skin Contact</u>: Wash off with soap and plenty of water. Consult a physician.
<u>Spills</u>: Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Evacuate personnel to safe areas. Beware of vapors accumulating to form explosive concentrations. Vapors can accumulate in low areas. Small contained spill: wearing appropriate PPE, collect with non-combustible absorbent material, (e.g. sand, earth, diatomaceous earth, vermiculite) and place in container. Dispose in Hazardous Chemical Waste. Large spills: Evacuate area and call 911 (Haz Mat).</td></tr>
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