Forensic Hair Examinations

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1 INTRODUCTION

This document describes the procedures for the microscopic examination, identification, and comparison of hair evidence, the conclusions that can be reached, as well as the steps taken to prepare a hair for subsequent DNA analysis. These procedures apply to hairs that have been previously recovered from evidentiary items and have been mounted on glass microscope slides.

This document specifies the procedures for the typical examinations performed on hair evidence. The case scenario or contributor request(s) may result in a different examination approach. In those instances, information will be recorded in the case notes stating which examinations will be performed or omitted (e.g., evidence will only be examined for pubic hairs), as well as the rationale or where the information for the rationale is recorded (e.g., incoming communication, communication log).

2 SCOPE

This document applies to hair examiners in the Trace Evidence Unit (TEU).

3 EQUIPMENT

- Comparison microscope, magnification range approximately 40x to 600x
- Stereobinocular microscope, magnification range approximately 2x to 40x
- Permount® mounting medium
- Xylene substitute, Xyless, or Xylene®
- Glass microscope slides and coverslips
- Pillboxes
- Forceps
- Spatulas
- Scissors
- Probes
- Scribes
- Microfuge tubes
- Adhesive pads

4 PROCEDURE

A. The microscopic examination of hairs requires the use of low magnification and high magnification.
B. A stereobinocular microscope can be utilized to screen the glass microscope slides at low magnification, and a high magnification comparison microscope is necessary to compare the microscopic characteristics of hairs, typically using a magnification range from 40x to 400x.

4.1 Documentation of Hair Evidence

The following are characteristics that may be used for identifying, classifying, and comparing hairs. This list is not all-inclusive; each characteristic may or may not be present in every hair. The type and number of characteristics recorded will be dependent on the characteristics present within the hair and the type of examination(s) conducted.

4.1.1 Macroscopic Characteristics

Color (in reflected light)
- White
- Blonde
- Red
- Brown
- Black

Shaft form
- Straight
- Arced
- Wavy
- Curly
- Twisted
- Tightly coiled

Shaft length (cm or in)

Overall shaft thickness
- Fine
- Medium
- Coarse

4.1.2 Microscopic Characteristics

Color (in transmitted light)
- Colorless (white)
- Blonde
- Red
- Brown
- Black

Natural pigmentation

Color treatments
- Dyes
- Bleaches/lighteners

Shaft diameter range (µm)

Medulla:

Absent
Continuous
Discontinuous
Fragmented
Opaque
Translucent
Relative width
Amorphous
Other (e.g., doubled, tripled)

Cuticle:

Present

Absent

Cuticle thickness

- Thin
- Medium
- Thick

Outer cuticle margin

- Flattened
- Cracked
- Looped
- Irregular or other
- Smooth
- Serrated

Inner cuticle margin

- Distinct
- Indistinct

Cuticle color and clarity

- Natural
- Artificially treated
- Pigment in cuticle

Cortex:

Cellular texture

- Coarse
- Medium
- Fine

Ovoid bodies

- Size
- Distribution
- Abundance

Cortical fusi

- Size
- Shape
- Distribution
- Abundance

Pigment size

- Coarse
- Medium
- Fine

Pigment aggregation

- Streaked
- Clumped
- Patchy

Pigment density

- Absent
- Uniform
- Peripheral
- One-sided
- Sparse – Heavy
- Central or medial

Proximal ends:

Root present

- Telogen
- Catagen
- Anagen
- Sheathed
- Follicular tag
- Postmortem banding
- Putrid

Root absent

- Severed
- Decomposed
- Crushed

Distal ends:

Tapered tips (uncut)

Rounded or abraded

Square cut

Angular cut

Frayed

Split

Crushed

Broken

Singed

Cross-sectional shape:

Round	Triangular
Oval	Flattened

Shaft configurations:

Buckling	Shouldering
Convoluting	Undulating

Acquired characteristics:

Artifacts
- Nits or lice
- Fungal tunnels
- Insect bite marks
- Debris

Damage
- Environmental/chemical damage
- Crushed
- Burned/Singed
- Cut
- Broken
- Frayed
- Twisted

A. The presence or absence of hairs can be determined based on a microscopic examination of the glass microscope slides containing debris recovered from an item (or group of items) of evidence.
    1. The absence of hairs will either be recorded in the case notes with a statement such as “no hairs were found,” or the absence of any reference to hairs on an item of evidence implies that no hairs are present.
B. If animal guard hairs are present, and a determination regarding the type of animal can be made based on an examination of the microscopic characteristics, this information will be recorded in the case notes as appropriate.
    1. If the type of animal is not determined, the presence of animal hair will be recorded in the case notes as appropriate.
C. If human hairs are present, the characteristics of ancestry (European ancestry; African ancestry; and/or Asian or Native American ancestry) and somatic origin (body area) may be determined based on an examination of the microscopic characteristics of the hairs.
D. The following tables list characteristics observed when classifying hairs into ancestral groups and somatic origin.
    1. When a hair is classified into one of the following groups, the listed characteristics are observed unless otherwise noted.
    2. The characteristics in these charts are not all-inclusive.

Head Hair

	European Ancestry Head Hairs	African Ancestry Head Hairs	Asian or Native American Ancestry Head Hairs
Ancestral Characteristics	Oval cross-sectional shape; Even pigment arrangement	Flattened cross-sectional shape; Clumped pigment arrangement	Round cross-sectional shape; Patchy pigment arrangement
Somatic Origin Characteristics	Minimal observed diameter variation throughout	Regular observed diameter variation throughout	Little to no observed diameter variation

Pubic Hair

	European Ancestry Pubic Hairs	African Ancestry Pubic Hairs	Asian or Native American Ancestry Pubic Hairs
Ancestral Characteristics	Oval cross-sectional shape; Even pigment arrangement	Flattened cross-sectional shape; Clumped pigment arrangement	Round cross-sectional shape; Patchy pigment arrangement
Somatic Origin Characteristics	Coarse diameter through root; irregular observed diameter variation throughout; buckling; prominent medulla	Coarse diameter through root; irregular observed diameter variation throughout; buckling; prominent medulla	Coarse diameter through root; irregular observed diameter variation throughout; buckling; prominent medulla

Facial Hair

	European Ancestry Facial Hairs	African Ancestry Facial Hairs	Asian or Native American Ancestry Facial Hairs
Ancestral Characteristics	Even pigment arrangement	Clumped pigment arrangement	Patchy pigment arrangement
Somatic Origin Characteristics	Coarse diameter; triangular cross-section	Coarse diameter; triangular cross-section	Coarse diameter; triangular cross-section

E. The examiner will describe hairs which exhibit characteristics of more than one ancestry as exhibiting characteristics predominantly of the ancestry which contributed the majority of the observed microscopic characteristics.
    1. If a predominant ancestry cannot be determined, the examiner will describe the hair(s) as exhibiting mixed ancestral characteristics.
        i. The case notes will indicate a characteristic(s) from the non-predominant ancestral group(s) observed.

F. If the microscopic characteristics do not allow for ancestry determination to be made (e.g., white hairs, opaque hairs), the hair will not be classified with respect to ancestry.
G. The presence of human head hairs and pubic hairs and their characteristics of ancestry will be recorded in the case notes.
    1. The presence of human facial hairs and their characteristics of ancestry will be recorded in the case notes, as appropriate.
    2. These hairs can be identified individually or collectively for each evidentiary item or group of items.
H. Head hairs, pubic hairs, and facial hairs that also exhibit characteristics of another somatic origin may be considered a transitional hair.
    1. This will be recorded in the case notes and the case notes will indicate a characteristic(s) observed from another somatic origin.
I. If human hairs do not exhibit characteristics of head, pubic, or facial somatic origin, these hairs will be described as body hairs, hair fragments, or fringe hairs.
J. Body hairs are typically not classified to a specific ancestral group or somatic origin.
    1. If body hairs will be further described as exhibiting characteristics of a limb hair, axillary hair, chest hair, or eyebrow/eyelash hair then the characteristics observed in the hair to support further classification of body hairs will be recorded in the case notes.
    2. If body hairs will be classified to a specific ancestral group, then characteristics observed in the hair to support ancestral classification of body hairs will be recorded in the case notes.
K. Additional microscopic characteristics present in hairs will be recorded as appropriate.
L. Hairs with a stretched root may be described as exhibiting characteristics of having been forcibly removed.
M. Damage to hairs may be described as exhibiting characteristics of having been cut, broken, crushed and/or burned.
N. A hair may be described as artificially treated (e.g., dyed, bleached) or as exhibiting characteristics of artificial treatment.
O. A hair may be described as exhibiting characteristics of apparent decomposition (e.g., postmortem root banding).

4.2 Hair Comparisons

4.2.1 Suitability

A. A determination of the suitability for meaningful microscopic comparison purposes will be made for hairs examined.
    1. A statement will be added to the case notes when the determination is made that a hair is either of limited value or not suitable for meaningful microscopic comparison purposes.
B. Hairs that exhibit sufficient microscopic characteristics for comparison are suitable for meaningful microscopic comparison purposes.

1. These hairs can be compared to known hair samples and all possible conclusions can be reached.
    i. Typically, head and pubic hairs are the only human hairs that fall into this category.
    ii. Typically, guard hairs are the only animal hairs that fall into this category.

C. Hairs that exhibit fewer distinguishing microscopic characteristics for comparison (e.g., white head hairs, transitional pubic hairs) may be determined to be of limited value for meaningful microscopic comparison purposes.
    1. These hairs can be compared to known hair samples, however, only exclusion and inconclusive results can be reached from a comparison.

D. Hairs that do not exhibit sufficient microscopic characteristics are not suitable for meaningful microscopic comparison purposes.
    1. These hairs will not be compared to known hair samples.

E. When a known hair sample does not contain sufficient hairs to demonstrate the possible range of variation in microscopic characteristics (i.e., limited known sample) a determination will be made as to the suitability for meaningful microscopic comparison purposes.
    1. This determination will be recorded.

F. It may be possible to reach an inclusion conclusion if the microscopic characteristics in a questioned hair are represented in the hairs in the limited known hair sample.

G. If the microscopic characteristics in a questioned hair are microscopically dissimilar to the hairs in a limited known hair sample, an exclusion conclusion will be reported, along with a statement indicating that the known hair sample was of limited suitability.

H. If the microscopic characteristics in a questioned hair exhibit both similarities and differences to the hairs in a limited known hair sample, an inconclusive conclusion will be reported, along with a statement indicating that the known hair sample was of limited suitability.

4.2.2 Comparison Method

A. If comparisons of hairs are conducted, questioned hairs will be examined to determine suitability prior to the comparison to one or more known samples.
    1. This does not preclude the ability to preliminarily characterize the known hair sample(s) prior to the assessment of the questioned hair to identify questioned hairs that will undergo further examination and/or comparison (ex. target search).

B. Comparison microscopy will be used to compare all of the microscopic characteristics present in the questioned hair(s) to the hairs in the known hair sample.

C. The comparison process will involve a direct comparison of the questioned hair and the known hair sample along the entire length of the hair utilizing all of the microscopic characteristics which are present in the hair.
    1. Questioned human hairs will be compared to known human hair samples from the same somatic origin.

2. Questioned animal guard hairs will be compared to known samples of animal guard hairs.

D. There is no minimum number of microscopic characteristics necessary to reach a conclusion.
    1. All of the characteristics present in the hairs will be considered as part of the comparison process.

E. The presence, absence, appearance, and distribution of characteristics in the hairs being compared are important considerations.
    1. The variability of hair characteristics between individuals allows for meaningful conclusions.

F. A meaningful difference between a questioned hair and a known hair sample is defined as a characteristic that is found in the questioned hair that cannot be found in the known hair sample.

4.2.3 Hair Comparison Conclusions

4.2.3.1 Inclusion

A. An inclusion can be determined when all of the microscopic characteristics in the questioned hair are represented in the known hair sample.
    1. Hairs microscopically consistent with the source of a known sample, found on items related to the source of the known sample (based on case information provided by the contributor) will not be considered an inclusion.

B. If the questioned hair is microscopically consistent with the hairs in the known hair sample then the source of the known sample can be included as a possible source of the questioned hair.

C. When a microscopic comparison of human hairs results in an inclusion, the following statements will follow the reporting of the conclusion:

> *The comparison of microscopic characteristics in hairs does not constitute a basis for personal identification and the number of individuals who could be included as a possible source of a specific hair is unknown. The inclusion of an individual as a possible source of the hair by comparison of microscopic characteristics should be evaluated in conjunction with the DNA analysis report(s) [lab number w/ case record #] dated [date of DNA report(s)].*

D. When microscopic comparison of animal hairs results in an inclusion, the following statements will follow the reporting of the conclusion:

> *The comparison of microscopic characteristics in hairs does not constitute a basis for identification. Animals of similar breed and color may also exhibit the same microscopic characteristics.*

E. A notation will be placed in the relevant portion of the case notes recording an inclusion.

4.2.3.2 Exclusion

A. An exclusion can be determined when a meaningful difference is observed between the questioned hair and the known hair sample.

B. If the questioned hair is microscopically dissimilar to the hairs in the known hair sample then, based on the known hair sample provided, the source of the known hair sample cannot be included as a possible source of the questioned hair.
C. A notation will be placed in the relevant portion of the case notes recording a hair exclusion.
    1. The case notes will indicate a characteristic(s) observed which is microscopically dissimilar to the hairs in the known hair sample.

4.2.3.3 Inconclusive

A. An inconclusive conclusion can be determined either when a questioned hair exhibits both similarities and slight differences to the hairs in the known hair sample, the questioned hair is microscopically similar to the known hair sample but is of limited value for meaningful microscopic comparison purposes, or the known hair sample provided is of limited value for meaningful microscopic comparison.
B. When one of these conditions are met, then no conclusion can be reached as to whether the source of the known hair sample can or cannot be included as a possible source of the questioned hair.
C. A notation will be placed in the relevant portion of the case notes recording an inconclusive result of the hair comparison.
    1. If the conclusion is that the questioned hair exhibits similarities and slight differences, the case notes will indicate the slight difference(s) observed in the microscopic characteristics present.

4.3 Hair Blind Verifications and Verifications

4.3.1 <u>Blind Verifications</u>

A. All hair inclusions will be blind verified by a second qualified examiner.
B. At the discretion of the examiner or technical reviewer, other hair comparison conclusions may also be blind verified by a second qualified examiner.
    1. When other hair comparison conclusions are selected for blind verification, a notation will be made in the case notes identifying which hair was selected for blind verification.
C. When hair comparison conclusions are blind verified, the second qualified examiner will not know the conclusion of the primary examiner and will not have been consulted by the primary examiner during the examination and comparison process.
D. The primary examiner will identify the items to compare and request a blind verification.
    1. The blind verification will be assigned to a second qualified examiner.
E. When available, multiple known samples will be provided to the blind verifying examiner.
F. For blind verifications, notes supporting the conclusion of the blind verifier will be taken and added to the examination records.
G. Blind verifications will be recorded in Forensic Advantage (FA).

4.3.2 Verifications

A. Other hair examination and comparison conclusions may be verified by a second qualified examiner at the discretion of the primary examiner or technical reviewer (e.g., postmortem root banding, appearance of damage, similarities and differences).
B. The primary examiner will identify the item(s) and hair conclusion and request a verification.
    1. The verification will be assigned to a second qualified examiner.
C. Blind verifications and verifications will be recorded in FA.

4.4 Hairs for DNA Analysis

4.4.1 DNA Suitability

A. Human hairs associated to a known hair sample will be assessed for DNA examinations.
    1. Other hairs may be submitted for DNA analysis at the discretion of the examiner.
B. Nuclear DNA analysis may be conducted on hairs with roots in any growth stage.
C. Mitochondrial DNA analysis may be conducted on any human hair, whether or not a root is present.

4.4.2 DNA Assessment

The determination as to which type of DNA analysis to prepare a hair for may be based on a combination of the following:

A. Growth stage of hair.
B. Examiner discretion.
C. Case type and/or details.
D. Input from other units.
E. Results of DAPI staining (see below).

4.4.3 DAPI Staining Procedure

A. Hair will be removed from the glass microscope slide.
B. Hair will be soaked in Xylene® or Xylene Substitute to remove any adhering Permount® or adhering glass fragments.
C. Hair will be placed on a new glass microscope slide or in a well plate, and the root treated by immersing in 1-2 drops of NucBlue® Fixed Cell ReadyProbes® Reagent (DAPI) by Invitrogen™, or equivalent.
D. After a minimum of 5 minutes, hair will be transferred onto a separate glass microscope slide and mounted in glycerol, or equivalent, with a cover slip for viewing.
E. The root will be examined under a microscope with fluorescence capabilities utilizing a UV/DAPI filter cube at 100-200x magnification to determine if visible nuclei are present and to count them.

1. Nuclei counts will be captured in the exam notes as 0, 1-24, 25-49, 50-99, or ≥100.
2. Additionally, it will be noted for which DNA the hair is suitable for based on the thresholds below:
    i. Hairs ≥ 25 nuclei will be suitable for submission for nDNA analysis when recovered from criminal cases.
    ii. Hairs ≥ 100 nuclei will be suitable for submission for nDNA analysis when recovered from Collected Exploitable Materials (CEM).

F. The hair will be rinsed in ethanol or an equivalent solvent to remove any adhering glycerin.

4.4.4 Preparing Hairs for DNA Analysis

A. Hairs must be removed from the glass microscope slide for DNA analysis.
    1. Precautions will be taken so the questioned hair is not contaminated with foreign debris and/or fluids.
B. Prior to removing the hair, a photograph may be taken of the root end of the questioned hair.
C. The hair will be removed from the slide.
D. The hair will be rinsed in Xylene substitute or equivalent solvent to remove any adhering mounting media.
E. For nuclear DNA analysis, the root portion of the hair will be removed and placed in a clean microfuge tube or dry mounted on a glass microscope slide.
F. For mitochondrial DNA analysis, an approximately 2 centimeter portion of the proximal end of the hair, when available, will be removed and placed on an adhesive pad, in a clean microfuge tube, or another acceptable container.
G. The hair will be subdivided from the primary item from which the hair was recovered.
H. If a portion of the hair remains, it will be preserved on an adhesive pad, maintained on the original slide, or preserved in another acceptable container.

5 LIMITATIONS

The comparison of the microscopic characteristics in human hairs does not constitute a basis for personal identification. The inclusion of an individual as a possible source of the hair by comparison of microscopic characteristics should be evaluated by the contributor in conjunction with the conclusion of DNA analysis, when available. Hair examinations may be limited where a considerable length of time exists between the deposition of questioned hairs and the collection of known hair samples.

The comparison of the microscopic characteristics in animal hairs does not constitute a basis for identification. Animals of similar breed and color may also exhibit the same microscopic characteristics.

6 SAFETY

A. While working with physical evidence, laboratory personnel will wear appropriate protective attire.

B. Universal precautions will be followed.
C. No specific hazards are associated with the microscopic examination techniques performed.
D. Care should be exercised when using solvents such as Xylene substitute.

7 REVISION HISTORY

Revision	Issued	Changes
09	01/28/2022	Reformatted entire document. Updated hair characteristic list to correspond to updated training material. Added Section 4.2.2 A. Updated language to clarify throughout.
10	11/01/2023	Section 4.4 revised to include new process for DNA assessment of hairs, to include DAPI staining.
11	12/16/2024	Removed SBAU references, added 4.4.3 for DAPI staining procedure.