TRACE EVIDENCE COLLECTION / HAIR-LIKE FIBER EXAMINATION

19.1 PURPOSE:

A. To properly collect trace evidence (a.k.a. trace material), specifically hair-like fibers.
B. To identify human hair(s) in collected trace evidence and prepare human hair(s) for DNA analysis.

19.2 RESPONSIBILITY:

Forensic Science Examiner/Connecticut Career Trainee who has successfully completed training according to FB SOP-26 (Training Manual and Checklist).

19.3 SAFETY:

The appropriate measures for the proper handling of biohazardous materials, sharp instruments and chemicals will be used according to GL-02.

19.4 DEFINITIONS:

A. OTM: Other Trace Material
B. SEM: Scanning Electron Microscope
C. GSR: Gun Shot Residue
D. QRW: Quality Record Worksheet (all located in Appendix 1)
E. LIMS: Laboratory Information Management System

19.5 PROCEDURE: General Guidelines

A. Questioned trace evidence may be collected in the Forensic Biology Unit or in other units of the Laboratory as necessary and transferred to the Forensic Biology Unit for examination.

B. Trace evidence will be collected, examined and retained in such a manner as to prevent the loss or contamination of the evidence.

C. For homicide cases:
   1. Generally, hair-like fibers and other trace material will be collected, packaged, labeled appropriately (section 19.6) and returned to the submission packaging.
   2. The collected trace material will not be sub-itemized in LIMS.
   3. If the trace material needs to be retained, see section 19.7.
   4. The presence or absence of hair-like fibers and/or other trace material will be documented on the appropriate QRW.

D. For sexual assault cases:
   1. If present, hair-like fibers will be collected (sections 19.6 and 19.7), retained (FB SOP-02: Sexual Assault Evidence Collection Kit Examination) and documented on the appropriate QRW. The absence of hair-like fibers will also be documented.
   2. The presence or absence of trace material, other than hair-like fibers, will also be documented on the appropriate QRW.

E. For other cases:
   1. Hair-like fibers and other trace material will not be collected. Therefore, no sub-itemization is necessary.
   2. The presence or absence of hair-like fibers and/or other trace material will be documented on the appropriate QRW.

F. See section 19.12 for report writing.

19.6 PROCEDURE: Trace Evidence Collection and Packaging (when necessary)

A. Trace material may be packaged in layers, with the primary packaging (paperfold, "sticky note", glassine bag, for example) containing the collected material and the secondary packaging (envelope, sheet protector, for example) containing the primary packaging.

B. Transparent/translucent packaging is preferred as it may prevent loss while verifying contents. (See 19.7.D below). Secondary packaging may be sealed prior to verification only if the contents can be seen through all layers of packaging.

19.6.1 Materials:

Below is a list of common trace material collection and packaging supplies, however, other suitable collection and packaging materials may be used, as case appropriate.

A. For collection:
   1. Forceps
   2. "Sticky notes"
   3. Lint roller
   4. Trace scraper (various sizes)
   5. Forensic trace collection vacuum
   6. Blade/scalpel

B. For packaging:
   1. Paper for paperfold (see paperfold addendum)
   2. Glassine bag
   3. Sheet protector (various sizes)
   4. White butcher paper (preferred)
   5. Trace tin (various sizes)
   6. Vacuum filter
   7. Envelope (various sizes)
   8. Glass microscope slide and cover slip (various sizes)

19.6.2 Collection by Picking

A. Clean forceps according to FB SOP-1, section 1.5.1 (cleaning utensils and laboratory area).

B. Pick visible hair-like fibers/OTM from the submission with the forceps. Note: Do not collect any hair-like fibers that are interwoven into the fabric since it is a sign that the hair-like fiber was not recently transferred/deposited.

C. Place the collection into appropriate primary packaging. Label this packaging with the Lab #, submission #/item #, description and initials. Place the primary packaging in a secondary package and label it similarly to the primary packaging.

D. After verification of the collected/retained trace material, (section 19.7 below), seal the secondary packaging and initial the seal.

19.6.3 Collection by Lifting

A. The adhesive side of a "sticky note" can be used to collect hair-like fibers/OTM from a smaller submission.
   1. The note may be folded upon itself. Place into the appropriate primary packaging.
   2. Label this packaging with the Lab #, submission #/item #, description and initials.
   3. Place the primary packaging in a secondary package and label it similarly to the primary packaging.
   4. After verification of the collected/retained trace material, (section 19.7 below), seal the secondary packaging and initial the seal.

B. A standard lint roller may be used to collect hair-like fibers/OTM from a larger submission.
   1. Remove the cover and set aside.
   2. Clean the roller top and handle according to FB SOP-1, section 1.5.1 (cleaning utensils and laboratory area).
   3. Discard the topmost layer of roller sheets.
   4. Roll the evidence, collecting the topmost removable trace material/debris.
   5. When the tackiness of the roller sheet diminishes, tear off the sheet and continue rolling the submission with the next sheet on the roll. Repeat as necessary.
   6. Place the used roller sheet(s) in a sheet protector, maximum two per protector. Sheets must be separate and apart from each other. Heat seal between the two roller sheets in the sheet protector and the sheet protector opening. Initial the heat seal(s). Label the sheet protector with the Lab #, submission #/item #, description and initials.
   7. The collected/retained trace material will be verified according to section 19.7 below.
   8. Clean the roller top and handle again according to FB SOP-1, section 1.5.1 (cleaning utensils and laboratory area) and replace the cover.

19.6.4 Collection by Scraping

A. Clean an appropriately-sized scraper or scalpel, according to FB SOP-1, section 1.5.1 (cleaning utensils and laboratory area).

B. In a contained area, hold the evidence above the clean white butcher paper and scrape the trace evidence from the submission onto the paper. Note: White butcher paper is preferred over brown craft paper because it is less likely to shed paper fibers during the collection process.

C. Use the scraper edge to gather the trace material into a pile on the clean paper. A paperfold may be made from this paper by cutting the paper around the piled trace material into a manageable size with a sharp blade/scalpel. Other types of primary packaging may be used as well.

D. Label the primary packaging with the Lab #, submission #, item #, description and initials. Place the primary packaging in a secondary package and label it similarly to the primary packaging.

E. After verification of the collected/retained trace material, (section 19.7 below), seal the secondary packaging and initial the seal.

19.6.5 Collection by Vacuuming

A. Clean the vacuum unit, hoses and cords according to FB SOP-1, section 1.5.1 (cleaning utensils and laboratory area).

B. Remove an appropriately sized vacuum filter from its sealed integrity packaging and attach it to the vacuum hose.

C. Vacuum an area of the submission for hair-like fibers/OTM, documenting the location. Change the filter as necessary, depending on the capacity of the filter. Continue to vacuum an area and document its location until all areas of interest are vacuumed.

D. Label the filter with the Lab #, submission #/item #, description, area of collection and initials. Place each filter in a secondary package and label it similarly to the filter.

E. After verification of the collected/retained trace material, (section 19.7 below), seal the secondary packaging and initial the seal.

19.6.6 Collection of SEM samples for GSR

Collect samples for SEM analysis according to FLIN SOP-07 (SEM sample collection on clothing for GSR analysis) located under Chemistry/Instrumentation-GSR.

19.7 PROCEDURE: Verification and Retention of Trace Samples

The trace evidence collected in the Forensic Biology Unit will be verified for the correct labeling and content by a second analyst (however titled) prior to its final packaging and disposition.

A. The samples collected from the evidence will be sub-itemized in LIMS according to GL-4 (LIMS/JusticeTrax) and FB SOP-1, section 1.5.5 (Examples of evidence/sample itemization in LIMS).

B. The type of trace evidence collected and retained (debris, mineral matter/material, other trace material, for example) and the general number of hair-like fibers present (at least three (3) hair-like fibers /greater than three (3) hair-like fibers, for example) will be documented in LIMS in the 'Notes' area associated with the created sub-item.

C. In LIMS, transfer the trace sub-items to the appropriate intended storage location (see 19.7.F below).

D. The LIMS transfer sheet will be printed. A second analyst will then view the collected/retained trace material and verify that the contents and labeling agree with the LIMS information.
   1. If in agreement, the second analyst will initial and date the LIMS transfer sheet.
   2. If the second analyst discovers a simple discrepancy in documentation, then the appropriate correction(s) will be performed.
   3. If the second analyst discovers a discrepancy greater than the above, then the Unit Lead will be informed, the root cause will be determined and corrected. Further appropriate action may be taken by the Unit Lead.
   4. The initialed and dated transfer sheet (or copy) will be retained in the appropriate case jacket(s).

E. After successful verification of the collected/retained trace material, seal the secondary packages and initial the seals.

F. Place the samples in the correct long term storage location:
   1. For LIMS 'Trace storage – retained trace' samples:
      Place the collected trace material in a larger manila envelope (approximately 9" x 12"), label with the Lab ID#, incident town, item # and examiner's initials. Seal with tape, initial the seal and place in trace storage.

   2. For LIMS 'Freezer storage' samples:
      If the presence of blood-like material or other body fluid-like material is suspected, place in a plastic bag, label with the Lab ID# and examiner's initials. Heat seal, initial the seal and place in freezer storage.

19.8 PROCEDURE: Examination of Questioned Trace Evidence/Identifying Human Hairs

19.8.1: Materials:

A. Stereomicroscope
B. Compound microscope
C. Glass microscope slides
D. Cover slips
E. dH_2O
F. Disinfecting solution
G. Digital imaging device

19.8.2: Procedure:

A. Record the lot numbers of all reagents/solutions used on the appropriate QRW.

B. Digital images may be used to aid in the examination/documentation process.

C. For collected trace material:
   1. Examine the collection macroscopically and stereoscopically, separating out any hair-like fibers.
   2. Return the remaining portion of questioned trace material to its packaging to be retained at the Laboratory.
   3. Assign each chosen hair-like fiber a number.
   4. Record the approximate length (in centimeters), color and texture of each hair-like fiber on the appropriate QRW. (Note: Some animal hairs may be identified stereoscopically. Tissue-like material may be observed stereoscopically. Examination at a higher magnification is usually necessary.)
   5. Choose the appropriate glass microscope slide and cover slip based on the length and morphology of the hair-like fiber evidence and parameters of the microscope.
   6. Label each glass microscope slide with the Lab #, submission #/item #, hair-like fiber # and initials.
   7. Temporarily mount each of the chosen hair-like fiber(s) in dH_2O, or other appropriate medium. Each hair-like fiber should be mounted separately, each with its own cover slip. The ends should be easily located.

D. For evidence that consists of hair-like fibers previously mounted on glass microscope slides with a permanent mounting medium:
   1. Assign each hair-like fiber a number.
   2. Examine the mounted specimens macroscopically and stereoscopically.
   3. Record the approximate length (in centimeters), color and texture of each hair-like fiber on the appropriate QRW. (Note: Some animal hairs may be identified stereoscopically.

Tissue-like material may be observed stereoscopically. Examination at a higher magnification is usually necessary.)

E. Examine each mounted hair-like fiber with a compound microscope at ~ 100x magnification.

F. The examiner will determine if each numbered hair-like fiber is a hair and whether the hair is of animal origin or human origin.

G. The examiner will document observations that support the identification conclusion of each hair-like fiber on the appropriate QRW.
   1. Examples of the hair documentation may include descriptions of root features, color features, cortex features, pigment features, cuticle (scale) features, tip features, medulla features or gross morphological features. The examiner will use their knowledge, training and experience to identify and characterize these features.
   2. A digital image (~100x) may be substituted for the written description.

H. The examiner will document the presence of a root (i.e. hair) or the absence of a root (i.e. hair fragment) for each human hair on the appropriate QRW.

I. The presence or absence of tissue-like material on the root will be recorded on the appropriate QRW for each human hair.

J. If deemed appropriate, the somatic body area and other microscopic characteristics of human hairs may be documented on the appropriate QRW.

K. The examiner will choose the most suitable human hairs for DNA analysis. (See sections 19.9.2 and 19.9.3.)

L. For mounted hair-like fibers not chosen for DNA analysis:
   1. Each temporarily mounted hair-like fiber may remain on the glass microscope slide. Allow the mounting medium to air dry and secure the cover slip with clear tape. Alternatively, each may be removed from its mounting and placed in the appropriate primary packaging.
   2. Permanently mounted trace material may remain on the glass microscope slide.
   3. Place the primary packaging in a secondary package and label it similarly to the primary packaging.
   4. After verification of the collected trace material, (section 19.7 above), seal the secondary packaging and initial the seal.

19.9 PROCEDURE: Preparing Hairs for DNA Analysis

19.9.1: Materials:

A. Sterile microcentrifuge tube(s)
B. Sterile scalpel(s)
C. Forceps
D. HistoClear or other appropriate solvent
E. Diamond scribe
F. Hood
G. KimWipes
H. Stereomicroscope

19.9.2: Nuclear DNA (nDNA) Analysis:

A. The examiner will use their experience and training to choose the most suitable hairs for nDNA analysis. These human hairs may be the hairs that possess the greatest amount of tissue-like material at the root and/or possess macroscopic morphological characteristics of interest.

B. The chosen human hair(s) will be itemized (See 19.11 *Itemizing*, below) on the appropriate QRW.

C. The root portion of each of the chosen human hair(s) will be photo-documented using an appropriate digital imaging device.

D. Remove the hair from the mounting (when necessary).
For permanently mounted human hairs:
1. Under a hood, crack the cover slip along the length of the hair with a diamond scribe.
2. Apply just enough HistoClear (or other appropriate solvent) to fill the crack and allow time for the medium to soften.
3. Use forceps to gently pry the hair out of the softened medium.
4. Remove any remaining medium from the hair by soaking it in additional HistoClear on a clean glass microscope slide and dabbing it with a KimWipe.

E. The examiner will confirm the length, in centimeters, of the human hairs(s) that are chosen for DNA analysis.

F. The examiner will excise the root portion of the hair.
1. Using a forceps, gently hold the root portion of the hair.
2. Use a sterile scalpel to slice the shaft above the held root portion.
3. Using the forceps, place the root portion in a sterile microcentrifuge tube.
4. The tube will be labeled with the Lab#, Item # and examiners initials. (See 19.11 *Itemizing*, below).
5. The tube will be placed in a plastic zip bag labeled with the Lab #.

G. The remaining shaft will be retained separately in appropriate packaging for possible future mtDNA analysis. The package will be labeled with the Lab#, Item # and examiners initials. (See 19.11 *Itemizing*, below).

H. After verification of the collected trace material, (section 19.7 above), seal the secondary packaging and initial the seal.

I. Transfer samples to the DNA analysis, or to a mutually accessable freezer storage location, as appropriate. See also section 19.7.F.

19.9 3: Mitochondrial DNA (mtDNA) Analysis

A. If the nDNA analysis of a human hair root does not yield significant results, the retained shaft portion of the hair may be forwarded to the DNA Unit, in its entirety, for mtDNA analysis.

B. If a chosen human hair does not possess tissue-like material at the root or does not have a root (i.e. hair fragment), the entire hair/hair fragment will be packaged appropriately with the package labeled with the Lab#, Item # and examiners initials. (See 19.11 *Itemizing*, below).

C. After verification of the contents, (section 19.7 above), seal the secondary packaging and initial the seal.

D. Transfer samples to the DNA analysis, or to a mutually accessible storage location, as appropriate. See also section 19.7.F.

19.10 PROCEDURE: Examination of Known Hair Samples

A. Known hair samples may be examined when deemed appropriate for the case scenario or when a biological known is needed for DNA comparison purposes.

B. Examining the microscopic morphology of the contents of the known hair sample is not necessary to designate the contents as human hairs.

C. Collecting human hair roots for DNA comparison purposes:
   1. Document the contents:
      a. Photo-document the entire contents of the known hair sample.
      b. Note the approximate number of hairs and their macroscopic morphological characteristics such as length, color and texture on the appropriate QRW.
   2. Examine the root ends of the hairs stereoscopically. It may be possible to identify tissue-like material on the hair roots. If tissue-like material cannot be discerned stereoscopically, then the hairs may be temporarily mounted on glass microscope slide for viewing under higher magnification.

3. Choose approximately five to ten hairs with tissue-like material and photodocument the root portions at the appropriate magnification.
4. The examiner will excise the root portions of the hairs (see 19.9.2.D above) and place all root portions into one (1) sterile micro-centrifuge tube.
5. The tube will be labeled with the Lab#, Item # and examiners initials. (See 19.11 *Itemizing*, below)
6. After verification of the contents, (section 19.7 above), seal the secondary packaging and initial the seal.

19.11 PROCEDURE: Itemizing

A. A trace evidence collection is given a sub-item number and documented in LIMS, according to FB SOP-1 section 1.5.5 and GL-04 (example: #1S1). Trace materials removed from the collection, further examined and not chosen for DNA analysis will remain part of the trace collection and labeled identically, however packaged.

B. Human hairs that are chosen for DNA analysis from that trace evidence collection are further sub-itemized (example #1S1-1).

C. A root portion that possesses tissue-like material that is chosen for nDNA analysis will be labeled with an asterisk (example: #1S1-1*) and considered a DNA consumption issue if an arrest has been made. The shaft portion from which that root has been removed and retained for potential mtDNA analysis, will remain itemized as above (example: #1S1-1).

19.12 PROCEDURE: Report Writing:

A. General Guidelines (Homicides and Sexual Assault cases):
1. If hair-like fibers are present, their collection and disposition will be specified in the Forensic Biology or DNA report.
2. The presence of trace material (i.e. not collected) will be specified in the Forensic Biology or DNA report.
3. Only when a trace examination is requested on the Request for Analysis form (SOP-ER-02) will the absence of trace material be included in the report.

B. General Guidelines (Other Cases):
1. The presence of hair-like fibers and/or other trace material will be specified in the Forensic Biology or DNA report.
2. Only when a trace examination is requested on the Request for Analysis form (SOP-ER-02) will the absence of trace material be included in the report.

C. Report Wording: Examination Method (as appropriate):
1. All examinations were conducted macroscopically unless otherwise noted.
2. All examinations were conducted microscopically and/or macroscopically.
3. All examinations were conducted macroscopically and microscopically.

D. Report Wording: Trace/Hair-like fiber Collection/Disposition
1. The report may include statements such as:
   a. Hair-like fibers and other trace material were noted on/in [ ].
   b. No hair-like fibers were noted on/in [ ].
   c. No trace material was noted on/in [ ].
   d. Trace material/hair-like fiber(s) was/were collected/removed from [ ] and returned to the submission/item packaging.
   e. Trace material/hair-like fibers, designated as item #1S1, was/were retained at the Laboratory.
   f. Trace material/hair-like fibers, designated as item #1S1,was forwarded for further examination.

E. Report Wording: Hair Identification/Disposition
1. A competent hair examiner from the Laboratory or another ASCLD accredited laboratory may act as the Technical Reviewer of a report which includes hair identifications.

2. The report may include statements such as:
   a. Human hairs were observed in item #1S1.
   b. No tissue-like material was observed on these hairs.
   c. Tissue-like material was noted on the root portion of some of these human hairs.
   d. The root portions of these human hairs were forwarded to the DNA Unit for nuclear DNA analysis.
   e. The shaft portions of these human hairs were forwarded to the DNA Unit for mitochondrial DNA analysis.
   f. Animal hairs were observed in item #1S1.

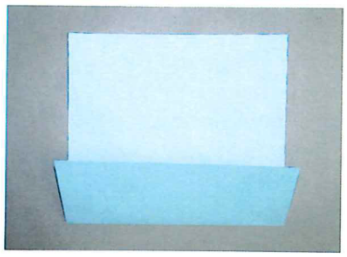
19.13 REFERENCES:

1. Hicks, J. Microscopy of Hairs: A Practical Guide and Manual, FBI Laboratory, 1977.
2. Saferstein, R. Forensic Science Handbook, Prentice Hall, 1982. Chapter 5: The Forensic Identification of and Association of Human Hair.
3. Saferstein, R. Forensic Science Handbook, Prentice Hall, 1982. Chapter 9: Foundations of Forensic Microscopy.

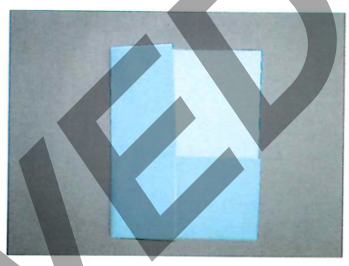
***Additional hair related articles are available.**

FB SOP-19 Trace Evidence Collection / Hair-like Fiber Examination - Addendum

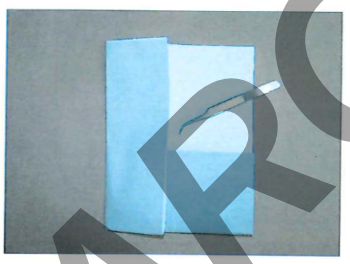
1. Fold 1/3rd of Width



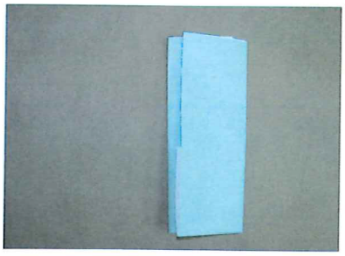
2. Fold 1/3rd of Length



3. Place Trace in Pocket



4. Fold Remaining 1/3rd of Length



5. Fold in Half

6. Fold Corners Under