TRACE EVIDENCE COLLECTION / HAIR-LIKE FIBER EXAMINATION

19.1 PURPOSE

A. To properly collect trace evidence (i.e. 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

Personnel qualified to perform Forensic Biology duties.

19.3 SAFETY

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

19.4 DEFINITIONS/ABBREVIATIONS

A. OTM: Other Trace Material
B. QRW(s): Quality Record Worksheet(s) (Appendix 1)
C. LIMS: Laboratory Information Management System
D. HLF(s): Hair-Like Fiber(s)

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 (19.6) and returned to the submission packaging.
   2. The collected/returned trace material will not be sub-itemized in LIMS.
   3. If the trace material is retained, see 19.7.
   4. The presence or absence of hair-like fibers and/or other trace material will be documented on the appropriate QRW(s).

D. For sexual assault cases:
   1. If present, hair-like fibers will be collected (19.6 and 19.7), retained (FB SOP-02: Sexual Assault Evidence Collection Kit Examination) and documented on the appropriate QRW(s). 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(s).

E. For other cases:
   1. Generally, hair-like fibers and other trace material will not be collected and returned or collected and retained, therefore, no sub-itemization is necessary.
      a. If the trace material needs to be collected, packaged and returned, see 19.5.C.
      b. If the trace material needs to be retained, see 19.7.
   2. The presence or absence of hair-like fibers and/or other trace material will be documented on the appropriate QRW(s).

F. See 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 if verifying contents is deemed necessary, as it may prevent loss. (see 19.7.D)

C. If the sample is to be retained, create the sub-item in LIMS.

The sub-itemized trace material being retained will immediately be held in a secure location. If transferred to a temporary storage location other than one's evidence locker, the transfer must be documented in LIMS. Evidence transferred into a temporary location does not need to be verified.

Upon transfer to the appropriate secure long-term storage location, the evidence will be verified. (see 19.7)

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. Bench paper*
5. Trace tin (various sizes)
6. Vacuum filter
7. Envelope (various sizes)
8. Glass microscope slide and cover slip (various sizes)

* Note: White paper is preferred over brown paper for trace examinations because it is less likely to shed paper fibers.

19.6.2 Collection by Picking

A. Clean forceps according to FB SOP-01 (Evidence Examination and Sample Collection Guidelines).

B. Pick visible hair-like fibers/OTM from the submission with the forceps. Note: It is suggested that any hair-like fiber(s) interwoven into the fabric may be a sign that the hair-like fiber(s) was not recently transferred/deposited and therefore may be avoided.

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

D. The collected/retained trace material will be verified according to 19.7.

E. Clean forceps again according to FB SOP-01.

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 #, item #, description and initials.
   3. Place the primary packaging in a secondary package and label it similarly to the primary packaging.
   4. The collected/retained trace material will be verified according to 19.7.

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-01.
   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, recording the number of sheets used.

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 #, item #, description and initials.
7. The collected/retained trace material will be verified according to 19.7.
8. Clean the roller top and handle again according to FB SOP-01 and replace the cover.

19.6.4 Collection by Scraping

A. Clean an appropriately-sized scraper or scalpel, according to FB SOP-01.

B. In a contained area, hold the evidence above clean bench paper and scrape the trace evidence from the submission onto the paper.

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 #, item #, description and initials. Place the primary packaging in a secondary package and label it similarly to the primary packaging.

E. The collected/retained trace material will be verified according to 19.7.

F. Clean the scraper and scalpel again (or discard scalpel after use), according to FB SOP-01.

19.6.5 Collection by Vacuuming

A. Clean the vacuum unit, hoses and cords according to FB SOP-01.

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 #, item #, description, area of collection and initials. Place each filter in a secondary package and label it similarly to the filter.

E. The collected/retained trace material will be verified according to 19.7.

F. Clean the vacuum unit, hoses and cords again according to FB SOP-01.

19.7 PROCEDURE: Verification and Retention of Trace Samples

The trace evidence retained in the Forensic Biology Unit will be verified for the correct labeling by a second analyst (however titled).

A. The trace retained from the evidence will be sub-itemized in LIMS according to GL-4 (LIMS).

B. The type(s) of trace evidence noted and retained will be documented on the appropriate QRW(s). (For example: hair-like fibers, debris, mineral matter/material, other trace material.)

C. In LIMS, electronically transfer each trace sub-item to the appropriate intended storage location. Locations include, but are not limited, to 'Trace Storage – retained trace' or 'Freezer Storage'.

D. The LIMS transfer sheet will be printed and a second analyst will verify that the labeling of the primary packaging, and secondary packaging, if present, agrees with the LIMS information. Due to the potential for sample loss, it is not recommended for the second analyst to view the contents of the primary packaging/collected trace material packet.
   1. If in agreement, the second analyst will initial and date the LIMS transfer sheet.
   2. If the second analyst discovers a discrepancy, then the appropriate correction(s) will be performed.
   3. The Unit Lead will then be informed and the root cause will be determined and corrected if possible. 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. Once verified, the envelope (or similar) containing the primary packaging with retained trace will be sealed and the seal initialed.

F. Place the sub-item in the appropriate location designated on the LIMS transfer sheet.
   1. For LIMS ‘Trace Storage – retained trace’ samples:
      Place each sealed trace material sub-item in a larger manila envelope (approximately 9” x 12”), label the larger manila envelope with the Lab ID#, initials and each sub-item # contained within. Seal with tape and initial the seal.

   2. For LIMS ‘Freezer storage’ samples:
      When the presence of blood or other body fluid-like material is suspected, place each sealed sub-item in a plastic bag, label the plastic bag with the Lab ID#, heat seal and initial the seal.

19.8 PROCEDURE: Examining Questioned Trace Evidence Collection for Hair-Like Fibers (HLFs).

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

B. Digital images of each step of the examination and each hair-like fiber examined may be very useful in determining the appropriate human hairs to forward for DNA analysis.

19.8.1: Materials:

A. Forceps
B. Probe(s)
C. Stereoscope
D. Glass microscope slides
E. Cover slips
F. dH_2O or other appropriate mounting medium
G. Compound microscope
H. Digital imaging device
I. Disinfecting solution

19.8.2: Procedure:

A. Macroscopically and stereoscopically examine the bulk trace evidence collection, using forceps and probe to separate out and set aside any hair-like fibers (HLFs). Return the remaining portion of questioned trace material to its packaging. Some trace evidence collections may consist only of hair-like fibers.

B. It is imperative that the examination of the HLFs be approached systematically to prevent sample loss.

C. Macroscopic/Stereoscopic Examination:
   1. Number each hair-like fiber.(i.e. HLF#1, HLF#2, HLF#3, etc).
   2. Record the approximate length (in centimeters), color and texture on the appropriate QRW(s).
   3. Stereoscopic examination of each HLF (optional):
      a. Some animal hairs may be identified stereoscopically.
      b. Tissue-like material may be observed stereoscopically. Examination at a higher magnification is usually necessary.
      c. Digital images will be captured of animal hairs or tissue-like material identified stereoscopically. A cm scale will be included.

D. For the following steps, the examination of each HLF will be completed before proceeding to the next HLF. Therefore, the macroscopic examination/documentation followed by the microscopic examination/documentation of HLF #1 will be completed before proceeding to HLF #2.

E. Prepare Each HLF for Microscopic Examination:

1. 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.

2. Label the glass microscope slide with the Lab #, item #, HLF# and initials.

3. Temporarily mount the HLF in dH_2O, or other appropriate medium. Each HLF should be mounted separately, each with its own cover slip. The ends should be easily located.

4. Once prepared, photograph the mounted HLF with a cm scale.

5. For multiple hair-like fibers previously mounted on glass microscope slides with a permanent mounting medium:
   a. Using a marker, draw a dot at one end of each HLF (root end, if present/discernible). Place a number next to each dot, thus assigning each hair-like fiber a HLF#.
   b. Record color, texture and approximate length (in centimeters), of each HLF on the appropriate QRW(s).
   c. Stereoscopic examination of each HLF (optional):
      i. Some animal hairs may be identified stereoscopically.
      ii. Tissue-like material may be observed stereoscopically. Examination at a higher magnification is usually necessary.
      iii. Digital images will be captured of animal hairs or tissue-like material identified stereoscopically. A cm scale will be included.

F. Microscopic examination for each HLF:

1. Examine the mounted hair-like fiber with a compound microscope, generally at ~100x magnification.

2. The examiner will determine if the HLF is a hair and whether the hair is of animal origin or human origin.

3. In addition, the examiner will determine the presence or absence of a root and tissue-like material if a root is present.

4. Photo-documentation:
   a. Digital images (taken under a 10x ocular or greater) of the root, tip and mid-section may aid in the examination, documentation and technical review process.

b. Images may be captured utilizing the Leica Application Suite (LAS EZ) capture software as follows:
   i. Turn on the microscope light and place the mounted sample on the stage.
   ii. To ensure a white background, the day light filter (DLF) toggle should be forward (towards operator), and the N16 toggle should be back (towards microscope body).
   iii. Open the LAS EZ program.
      - In the options drop down, select preferences.
      - Under the save images field select the black button with the ellipsis (…) to choose a folder in which to save your images.
      - Select (or create) the appropriate case images file, saved by year in the criminalistics folder on the lims drive.
      - The image is captured under the Acquire tab:
        - The automatic exposure setting (shutter icon) should be highlighted.
        - A white balance (shaded square icon) may be performed on the image.
        - Once the desired image is in focus on the screen, select the acquire image button.
        - The screen automatically advances to the browse tab, where the image is displayed on the main screen, as a thumbnail in the field at the bottom of the page and has been saved as a .jpg file to the selected case images file.
        - Right click on the thumbnail to rename (select rename current image option).
      - Additional information may be added under the Process tab:
        - A text box may be added with any desired case/item information under the annotate tab. The file name, date, and time of collection may also be added as text boxes to the image.
        - In the actions box, select merge to overwrite the original image with the annotated version (note: it is not necessary to generate an annotated image).

5. Images should be organized in the examination worksheets in a manner that can be easily followed by the case reviewers. For example, on one page or a series of consecutive pages group the photos as follows:
   a. An overview image of HLF#1 mounted on a glass microscope slide with appropriate labeling and a cm scale will be included.

b. The next image may be a stereoscopic image of HLF #1 with a cm scale.
c. Next, an image or series of microscopic images of HLF #1 may include root/tlm, shaft and tip.
d. Repeat for HLFs #2, #3, #4, etc.

6. The examiner will document observations that support the identification conclusion of each HLF# such as the presence of a cuticle, cortex and medulla on the appropriate QRW(s).

   Additional examples of hair documentation may include descriptions of root, color, cortex, pigment, cuticle (scale), tip, medulla and/or gross morphological features. The examiner will use their knowledge, training and experience to identify and characterize these features.

7. 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(s).

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

G. A second qualified examiner will confirm the identification conclusion.
   1. They may observe the actual HLF or may review the image(s) and description(s) (i.e. during the Technical Review).
   2. They will initial and date the appropriate QRW(s) to document the confirmation.

H. The examiner will choose the most suitable human hairs for DNA analysis.

I. For HLFs not chosen for DNA analysis:
   1. Each temporarily mounted HLF may remain on the glass microscope slide by allowing the mounting medium to evaporate and securing the cover slip with clear tape. Alternatively, each HLF may be removed from its mounting and placed in a separate package (usually a paperfold or glassine bag), sealed and labeled with the Lab #, item #, HLF # and initials. The individual HLFs (however packaged) will be returned to the original packaging whenever possible. Examined HLFs should never be returned to the packaging without proper labeling.
   2. Permanently mounted HLFs/trace materials 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. Document on the appropriate QRW(s) how these HLFs/trace materials are re-packaged. (Example: item #001-002 (trace material from hat) retained as 10 glass microscope slides plus the original one white paperfold).

5. The re-packaged retained trace material will be re-verified prior to long term storage to ensure the accuracy of the new packaging description.

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. Stereoscope

19.9.2: DNA Analysis:

A. The examiner will use their experience and training to choose the most suitable hairs for DNA 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. It is to be noted that Mitochondrial DNA analysis is not conducted at this Laboratory.

B. Generally, only the chosen human hair(s) will be itemized (see 19.11) on the appropriate QRW(s). (example: HLF#1 is chosen and further designated in LIMS as item #001-001-01, HLF#8 is chosen and further designated as item #001-001-02 in LIMS, etc.)

C. The root portion of each of these itemized human hair(s) will be photo-documented using an appropriate digital imaging device (if not previously photo-documented).

D. Remove the hair from its mounting:

For temporarily mounted human hairs

1. Slide the cover slip off the microscope slide.
2. Dry the slide/human hair with a clean KimWipe.

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.
5. If the hair is removed in its entirety, the examiner will confirm the length, in centimeters.
6. Alternatively, only the root portion may be removed. The remaining shaft will be appropriately documented and labeled.

E. Excise the root portion of the hair.
   1. Using forceps, gently hold the root portion of the hair. The hair may be placed onto the adhesive area of a "sticky note" for excision.
   2. Use a sterile scalpel to slice the shaft above the held root portion.
   3. Using the forceps, place the root portion into a sterile microcentrifuge tube.
   4. The tube will be labeled with the Lab#, item #. (see 19.11).
   5. Root portions with no consumption issue being forwarded for DNA analysis will not be verified at this time. These samples will be electronically transferred in LIMS and physically placed directly into a designated storage location for pending DNA extractions and added to the appropriate DNA batch spreadsheet.
   6. Root portions with a consumption issue will be placed in a plastic bag labeled with the Lab #. These samples will be verified and the bag heat sealed and initialed (see 19.7). These samples will be electronically transferred in LIMS and physically placed in a designated storage location.
   7. Remaining hair shafts being retained will be packaged accordingly, verified (see 19.7), electronically transferred in LIMS and physically placed in a designated storage location.

F. When these items are prepared to be sent to another Laboratory for analysis, the samples, in their entirety will be photo-documented and appropriately packaged. The appropriate packaging will have a Laboratory barcode label affixed. The labeling will include the Laboratory #, item # and detailed description of the contents and its origin. The examiner will place their initials on the packaging/seal.

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. The use of the submitting agency's labeling is appropriate to use in quotes to describe the sample, for example: "known head hair".

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(s).
   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 photo-document the root portions at the appropriate magnification.
4. The examiner will excise the root portions of the hairs (see 19.9.2.D) 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)
6. Enter description as "known head hair roots – person's name" (or similar) in LIMS.
7. Root portions with no consumption issue being forwarded for DNA analysis will not be verified at this time. These samples will be electronically transferred in LIMS and physically placed directly into a designated storage location for pending DNA extractions and added to the appropriate DNA batch spreadsheet.
8. Root portions with a consumption issue will be placed in a plastic bag labeled with the Lab #. These samples will be verified and the bag heat sealed and initialed (see 19.7). These samples will be electronically transferred in LIMS and physically placed in a designated storage location.
9. Remaining hair shafts being retained will be packaged accordingly, verified (see 19.7), electronically transferred in LIMS and physically placed in a designated storage location.

19.11 PROCEDURE: Itemizing

A. A trace evidence collection is given a sub-item number according to GL-04. 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 according to GL-04.

C. A root portion that possesses tissue-like material that is chosen for DNA analysis will be labeled with an asterisk according to GL-04 (LIMS) and considered a DNA consumption issue if an arrest has been made. The shaft portion from which that root has been removed will be retained and remain itemized as above (see 19.11.B).

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 #[ ], was/were retained at the Laboratory.
      f. Trace material/hair-like fibers, designated as item #[ ],was forwarded for further examination.

E. Report Wording: Hair Identification/Disposition
   1. A competent hair examiner from the Laboratory or another ASCLD/ANAB 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 #[ ].
      b. No tissue-like material was observed on these hairs.
      c. A human hair fragment was observed in item #[ ].
      d. No root and/or tissue-like material was observed on this human hair fragment.
      e. Tissue-like material was noted on the root portion of some of these human hairs.
      f. The root portions of these human hairs were forwarded to the DNA Unit for analysis.
      g. The shaft portions of these human hairs were retained at the Laboratory.
      h. Animal hairs were observed in item #[ ].

19.13 REFERENCES:

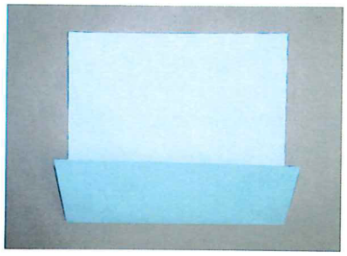
1. Li, Richard, Forensic Biology, Second Edition, CRC Press, 2015. Chapter 4: Sources of Biological Evidence, pp. 89-92 and pp. 93-95 (Figures 4.20-4.24).

2. Hicks, J., Microscopy of Hairs: A Practical Guide and Manual, FBI Laboratory, 1977.
3. Saferstein, R., Forensic Science Handbook, Prentice Hall, 1982. Chapter 5: The Forensic Identification of and Association of Human Hair, pp. 185-221.
4. Saferstein, R., Forensic Science Handbook, Prentice Hall, 1982. Chapter 9: Foundations of Forensic Microscopy, pp. 417-528.

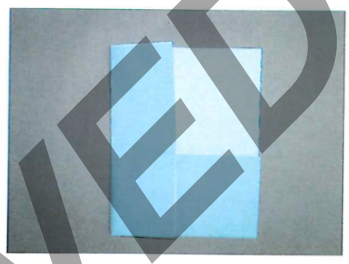
***Additional hair related articles are available.**

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

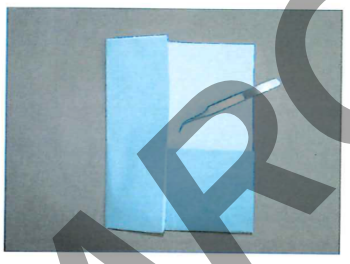
1. Fold 1/3rd of Width



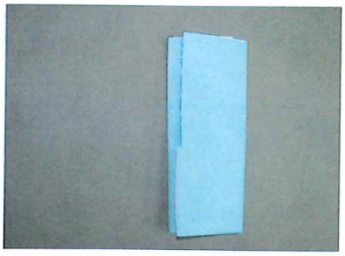
2. Fold 1/3rd of Length



3. Place Trace in Pocket



4. Fold Remaining 1/3rd of Length



5. Fold in Half

6. Fold Corners Under