PROCEDURES FOR HAIR ANALYSIS

Hair examination involves the meticulous **visual examination** and **searching** of articles of evidence for the presence of hair, which are mounted on microscope slides in mounting medium ("Cytoseal 280"). The analyst determines **species origin**, **body origin**, and **microscopic properties**. A microscopic comparison is then performed to determine possibilities of individual origin (humans) or species family origin (animals). An expert opinion is then rendered based on this analysis. After the hair is prepared by one of the above listed methods, the hair can be placed on the **comparison microscope** or **polarizing microscope** to determine that hairs **external and internal characteristics**. **Cross sectioning** techniques of hair shafts can be used to aid in this examination.

Once the analyst has identified these characteristics, he has the task of determining whether the hair is **human**, the following complex determinations must be made when possible:

1. Racial Origin (Caucasian, Negroid, Mongoloid and mixed racial origin).
2. Body Origin (Facial, Head, Pubic, Limb, Axillary and other body hairs).

When the hair is classified (regardless of race) as head hair or pubic hair, then the characteristics of the hair from the evidence are compared directly to known standards of hair from the individuals involved in the case. By comparing this characteristics, the analyst formulates a expert **opinion** based on the information collected from the microscopic comparison of the question(s) and\or known(s) hair(s). In order to formulate his\her opinion based on this comparison, an analyst will need an extensive knowledege, experience and training in forensic hair analysis.

When the hair is classified as **animal**, the examiner then should try to assign the hair as originating from a scientific family and if possible to a member of that family. After the animal hair is mounted on a microscope slide in a mounting medium ("Cytoseal 280"). The hair(s) is examined with the aid of a **comparison and\or polarizing microscope**. Scale casting techniques can be used by the analyst to aid the examination. The analyst will examine the **external and internal microscopic characteristics** to formulate his\her opinion. In order to formulate an opinion based on comparison, the analyst will require an extensive knowledge, experience and training in forensic hair analysis.

Visual Examination

After the item(s) of evidence are searched, the tapings and\or tins\envelopes containing the trace debris collected from the evidence are visually examined for the presence of hair. The analyst will use magnifying glass, magnifying glass lamp and\or **stereo microscope** (this instruments allows the user to examine trace evidence material at the magnifications of 6X to 50X, with a 3-D effect) to aid the analyst's examination. All hairs determined to be of suitable value for mounting are set aside for mounting on microscope slides for further examination.

Mounting hairs for Microscopic Examination

1. The hair(s) are placed on a clean microscope slide.
2. The mounting medium ("Cytoseal 280") is applied so that a thin film of the material will

totally cover the hair(s).

3. Then a cover slide is placed on top of the hair and mounting medium.
4. The hair(s) is ready for examination under a **microscope**(comparison and\or polarizing).

Comparison and Polarizing Microscopes

1. The slide is placed on the stage.
2. The objective which will give the analyst his\her desired magnification is selected.
3. The microscope is then setup for "Kohler Illumination" (applies to either microscope).
   A. Focus on the sample prepare on the microscope slide.
   B. Close the field diaphragm and focus its image in the field of view by racking the substage condenser up or down until the analyst gets a crisp image.
   C. Center the focused field diaphragm image by centering the condenser.
   D. Open the field diaphragm image until to just past the field of view.
   E. Remove one of the oculars and adjust the aperture diaphragm until the diaphragm occupies $^{2}/_{3}$ of field of view.
4. The object(s) is examined by the analyst, wether doing a side by side comparison and\or standard evaluation of the hair(s).

Human

1. Analyst determines if the hair(s) is human or animal with the aid of **microscope(s)**.
2. Once the hair(s) is determined to be human.
3. The race is determined for the hair with aid of **microscope(s)**, if the hair is a suitable sample.
   A. Caucasian.
   B. Negroid.
   C. Mongoloid.
   D. Mixed Racial.
4. Body origin (Head, pubic, limb, beard, etc.) with the aid of **microscope(s)**.
5. If the hair is head or pubic hair and of sufficient value. It can be compared to the know standards for the case with the aid of **comparison microscope**.
6. The following **opinions** are possible when stating the analyst's results of analysis:
   A. Not consistent with, or microscopically different.
   B. Consistent with.
   C. Inconclusive.
   D. Similarities and dissimilarities.
   E. Force Characteristics (IE., cut, pulled, burned).

Animal

1. Analyst determines if the hair(s) is animal or human with aid of **microscope(s)**.
2. Once the hair(s) is determined to be animal.
3. The type of hair(s) will be determined (guard or fur) with aid of **microscope(s)**.
4. Only guard hair(s) can be examined further (must have tip and base intact to be compared).
5. By examining the guard hair(s), the animals family [IE. leporidae (hares and rabbits),

Cervidae (Deer), Bovidae (cattle, goats and sheep)] and sometimes genus (eastern cottontail, mule deer, elk, bison, bighorn sheep) can be determined. The following factors are used to determine the family and possible genus is listed in species origin (IE. root, medulla, scale pattern, tip, color banding patterns > species origin) with aid of **microscope(s)**.

6. No further analysis can be conducted.

Species Origin\Hair Characteristics (External and Internal)

After the collection and\or mounting process, the macroscopic and microscopic properties of hair (internal and external characteristics) are studied, and developed using **Scanning Electron Microscope(SEM)**, **stereo microscope**, **polarizing microscope** and\or **comparison microscope** (magnifying powers range from 6X to 400X magnification depending on the **microscope** system(s) used);

1. ***Scales***
   A. Thickness
   B. Colors
   C. Scale patterns [IE. cerenal, spinous, imbricate, polygonal, hexagonal, and diamond].

2. ***Cortex***
   A. Pigment colors (IE. all shades of brown, black, blonde, red and combinations).
   B. Pigment size.
   C. Pigment shape.
   D. Pigment, interspacial relationship.
   E. Pigment distribution.
   F. Pigment clumping.
   G. Pigment pattern.
   H. Pigment pattern variations.
   I. Ovid bodies.
   J. Cortical fusi.
   K. Bleaching(natural, artificial).
   L. Amount of pigment.
   M. Chemical treatment (IE. dyes, bleaching, etc.).
   N. Banding (color changes).

3. ***Medulla***
   A. Patterns (IE. fragmental, continuous, discontinuous, latent, amorphous).
   B. Distribution (peripheral, cortical).
   C. Cellular size.
   D. Optical relief patterns (streaking).

4. ***Shaft***
   A. Size.
   B. Shape (IE. arch, buckling, straight, and curly).

C. Length.
D. Debris [cosmetics (powders, sprays), soil and blood].
E. Cross sectional shapes (round, oval, and flat).
F. Parasites (head lice, public lice, lice egg cases, and fecal material).
G. Damage [crushing, cutting (dull to sharp instrumentation determination), break and tearing (teasing, split ends, etc.), weathering(skeleton remains, etc.), burning (charred or singed determinations made), and water exposure(drowning).
H. Diseases.

5. ***Root***
A. Pigments.
B. Cortical fusi.
C. Follicular tagging (tissue adhering to root indicating forcible removal).
D. Atrophy (natural fallout).
E. Bulbous (length of root).
F. Shapes [spade (dog family and others), wine glass(deer family), bulb(human, primates, bear, others), and elongated (cat family and others), etc.
G. Growth stage (anagen, catagen, and telogen).
H. Force.

6. ***Tip***
A. Frayed (wearing from clothes, etc.).
B. Split.
C. Curled (natural or artificial).
D. Cut (dull, sharp, and razor).
E. Natural.
F. Broken.

A protocol is developed from the question hairs, known head and\or pubic hair standards for each and every item involved in the comparison process. This protocol is developed based on the examiners training and experience using the hair's characteristics. The protocol developed for the known\ question hair(s)are used to compare the known hair standards to the question hairs.

Cross Sectioning

1. ***Wire loop technique***.
A. Insert a wire loop through a hole in a black plastic plate.
B. Place approximately 3 - 5 strands or rayon yarn through the loop and pull about $^{1}/_{4}$ of the yarn through the hole in the plate.
C. Place the hair in question through the hole until the hair in question is aligned in the hole to both sides of the plate.
D. Using a sharp razor blade cleanly slice the yarn at the surface of the plate (on both sides).
E. Mount the section of black plastic plate on a glass microscope slide.
F. Use either a permanent mounting medium ("Cytoseal 280"), cargille refractive index liquid or air as a mounting medium for the plate.

G. Place cover slide over the slide. If air is the desired mounting medium, use tape to secure the edges of the cover slide.

H. The cross section is ready for examination on a **polarizing and\or comparison microscope.**

2. ***Microtone method.***

A. The hair is placed into a mold.

B. The epoxy, wax or resin material is placed in mold with the hair.

C. After the liquid solidifies around the hair, the mold with the hair is trimmed down to workable size.

D. The mold unit is placed into the microtone and cut into sections.

E. The cut sections are placed onto a glass microscope slide(s).

F. Use either a permanent mounting medium ("Cytoseal 280"), cargille refractive index liquid or air as a mounting medium for the plate.

G. Place cover slide over the section. If air is the desired mounting medium, use tape to secure the edges of the cover slide.

H. The cross section is ready for examination on a **polarizing and\or comparison microscope**.

3. ***Polymer film technique.***

A. A small section of standard sterile plastic drop cloth material is cut into approximately 1" to ½" sections.

B. One (1) section of the film is placed onto a microscope slide.

C. The hair is placed on top of the film on the microscope slide.

D. Another piece of the sectioned film is placed on top of the hair. Then a cover slide is placed on the top piece of film.

E. The microscope slide containing the film\hair\film\cover slide is placed onto a hotplate with the microscope slide coming in contact with the heated surface (low setting for the hot plate).

F. This assembly remains in contact with the until the film turns from the normal cloudy state to a clear appearance. The hair(s) is incased in the polymer film.

G. After the assembly has cooled, a sharp razor blade is used to trim and section the assembly into to small sections.

H. The sections are placed onto a clean microscope slide.

I. Place a cover slide over the section(s).

J. Use tape to secure the edges of the cover slide.

K. The cross section is ready for examination on a **polarizing and\or comparison microscope.**

Scale casting

1. ***Polaroid film coat method.***

A. Take a clean glass microscope slide.

B. Apply the Polaroid film coat to the surface of the slide.

C. Lay the hair(s) into the film coat on the slide. Note tip and root on slide.

D. Allow the hair(s) and film coat to dry for several minutes.

E. Remove the hair(s) to leave the scale cast(s).

F. The scale cast can be examined with the aid of the **comparison and\or polarizing microscope.**

2. ***Norland Optical adhesive method.***

A. Place one to two drops of Norland Optical adhesive on a clean glass slide.

B. Spread the liquid evenly on the slide.

C. Align the hair(s) in the adhesive. Note the tip and root on slide.

D. Cure by placing under long wave ultraviolet light (320 - 400 nm) for approximately twenty minutes.

E. After the curing process, remove the hair(s) to leave scale cast(s).

F. The scale cast can be examined with the aid of the **comparison and\or polarizing microscope.**

Note: Technique is generally used only on animal hair examination.

Preparation of Hair Root for DNA Analysis

1. Locate root end of designated hair on microscope slide. Using a scribe, puncture cover slip around the root. Add drop of xylene to root area.
2. Remove pieces of cover slip. Using a razor with a new blade, cut the root end from the hair.
3. While holding the hair with sterile forceps, rinse root with xylene.
4. Rinse root in 100% ethanol.
5. Rinse root with a thorough rinse in distilled water.
6. Place hair root end into a labeled 2.2 ml spin-ease tube. Place into labeled manilla envelope.

Instrumentation, Equipment and Tools used for Hair Analysis

- Comparison microscope with polarizing and fluorescent light capabilities.
- Stereo microscope/camera system.
- Polarizing microscope.
- Scanning Electron Microscope (SEM).
- Magnifying Glass (Hand held or Lamp).
- Tweezers, probes, scalpels, scrapping tools, etc.
- Tape used for collecting and searching (Methanol soluble).
- Containers (envelopes, tins, plastic bags).
- Vacuum pump and Filtration system (Methanol).
- Mounting Medium ("Cytseal 280" by Stephens Scientific, xylene, air, etc.).
- X-sectioning tools [Microtone and mounting medium (epoxy or resin solution), razor blades, rayon yarns, black x-section plates, hot plate, polymer film (olefin)].
- Scale casting materials ("Polaroid" film coating), Hot plate.
- Microscope slides, cover glass slips, cardboard mailers, slide tray(s) and microscope storage boxes.
- Lab coat, protective gloves (surgical type gloves), lab glasses.
- Glassware (beakers, flasks, etc.).
- Portable fume hood.

CONCLUSIONS

1. Microscopically consistent with > Therefore, could have originated from subject.
   Could have originated from> The question hair(s) did come from that individual or another individual, who has these same hair characteristics.

2. Not consistent with(found to microscopically different)> Therefore, did not originate from subject.
   Did not originate from> The question hair(s) characteristics are different from the known hair characteristics. This question hair can be eliminated from coming from this known individual(s).

3. No transfer> No hair(s) was found on items belonging and\or associated with the victim\suspect, which was suitable for microscopical comparison purposes or not microscopically consistent with the hair standards from the suspect\victim.

4. No proper standards> There was not a sufficient known hair standard submitted or no known hair standard(s) submitted at all.

5. Signs indicative ***of force, being cut, burned, etc.***> The known and\or question hair(s) have these characteristics of this type of treatment to the hair.

6. Not suitable for any further comparison purposes> The question hair(s) comes from a body area other than the head or pubic region of the body. It could also mean the question hair is of insufficient quality and\or quantity to compare to a known head hair or pubic hair standard(s).