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Letter to the Editor—Scientific Working Group on Materials Analysis Position on Hair Evidence

Hair evidence is frequently encountered in criminal investigations because hairs are readily lost from both victims and suspects during criminal activity. Hairs are easily transferred, easily detected and recovered, and are very durable. Most importantly, hair evidence can supply investigative leads and provide a direct association between the crime and the criminal, which, of course, should be the goal of any forensic investigation.

The first step in any hair examination is to separate the hairs from the submitted evidence. Some cases may result in the collection of relatively few hairs, while other cases may result in the recovery of hundreds or even thousands of hairs when numerous items or items such as vacuum sweepings or bedding are submitted. When hairs are first isolated from an item of evidence, a microscopical examination can initially determine which hairs may have evidential value out of the myriad of hairs that are often recovered. An example of hairs having potential evidential value would be those found on the victim's clothing or in their environment that are microscopically dissimilar to their own hairs. Hairs deemed to have potential evidential value will then be considered for further detailed microscopical comparison and possible DNA analysis. This initial screening step must be done by examining the hairs that are collected during the investigation of a crime using a good quality microscope capable of high magnification and conducting a brief yet detailed microscopical comparison. A detailed microscopical examination is necessary to isolate these hairs because it is common for hairs from different individuals to look the same macroscopically but differ microscopically. Unfortunately, many laboratories have adopted screening methods which rely solely on low level evaluations of hairs with a stereomicroscope to isolate those for DNA analysis that are macroscopically dissimilar in color and shape from the submitted known hairs. Screening methods used by other laboratories are even less discriminating in that they simply choose all hairs with root tissue and run nuclear DNA analysis on them regardless of their appearance. Ultimately, any procedure which does not include a detailed microscopical examination will likely result in hairs of probative value being overlooked or in time and money being wasted conducting DNA analysis on nonprobative hairs.

Once hairs of interest are isolated, the microscopical analysis of these hairs can provide investigative information such as the racial characteristics of the hair donor, the somatic origin of the hair, the growth phase of the root, and the presence or absence of artificial treatment or damage to the hair. Some of these features can aid in providing a physical description of a suspect and others can provide reconstructive information concerning certain activities that may have occurred during the commission of the crime. For example, hairs that have anagen roots have been removed from the scalp with some force; therefore, the presence of hairs at a crime scene that have anagen roots may indicate that there was a struggle between the hair donor and another individual.

The next step in a hair examination is often a direct microscopical comparison between the questioned hair(s) and known hair samples collected from the victim(s) and suspect(s). This comparison may be used to associate the individuals involved in a crime to the crime scene or to each other. This is possible because the microscopical characteristics observed in the hair of one individual are usually very different from those observed in the hair of another individual.

Once the microscopical comparison of the hairs is completed, a microscopical examination of the hair root will assess the potential for DNA analysis. Hairs will commonly lend themselves to either mitochondrial DNA (mtDNA) analysis or, if enough root tissue is present, to nuclear DNA (nDNA) analysis. The value of DNA analysis as a means to associate the criminal with the crime has been well established.

For the past 100 years, microscopical hair comparisons have been relied upon to provide possible associations or exclusions between a recovered hair and known sources of hair. This has been the case even though the microscopical comparison of hairs does not provide an absolute means of personal identification. Now with the advent of DNA typing methods, if enough root tissue is present to conduct nDNA analysis, the profile can be compared to a known nDNA sample from a suspect or victim and provide a near certain association. This provides the best possible link that can be made between a questioned hair and a known individual, and yields a virtually positive association to a single person. Additionally, if no suspects have been developed, the profile can be searched in the Combined DNA Indexing System (CODIS) in an effort to identify the individual that the hair originated from. Because of the strength of the nDNA testing, it is often considered to be a preferred and sufficient method for hair examination by many laboratories. However, nDNA alone cannot determine whether a questioned hair is indeed a human hair or a synthetic wig fiber, if it is a head hair or a pubic hair, a hair that has naturally fallen out or one that may have been forcefully removed, a hair exhibiting a decomposed root from a deceased individual, or a hair coated with blood or semen. Only a microscopical examination can determine these and many other attributes of hair evidence. Aside from this fact, the overwhelming majority of hairs found in forensic casework do not possess enough tissue to conduct nDNA analysis. When this is the case, mtDNA analysis is often successful. While mtDNA, like microscopical analysis of hair, cannot be used to unequivocally identify an individual, it can be used to exclude a large portion of the population as a possible donor of the hair and thereby provide very probative evidence. In this situation, the strongest possible association is made by first comparing the physical aspects of the hair through microscopy, then by comparing the genetic aspects of the hair through mtDNA analysis.

As mentioned previously, many forensic laboratories have eliminated the microscopical comparisons of hair in favor of utilizing only DNA analysis. Criticisms of microscopical hair comparisons have evolved from the inability of this technique to positively identify an individual and from recent publicity principally due to incorrect associations made by a few incompetent hair examiners. Errors regrettably occur in all scientific endeavors and should not detract from the information provided by any well-founded scientific discipline. Understanding the limitations of forensic hair examinations through appropriate and adequate training programs and quality control programs will greatly minimize these errors. Proper explanation of the limitations, meaning and significance of the microscopical hair identification and comparison will also provide a fair basis from which courts and juries can measure the value to the case and thereby minimize its erroneous use. Eliminating the microscopical examination of hairs is, in our opinion, irresponsible and unethical. In order to isolate the most probative hairs for

further examination, provide the best investigative information, and create the strongest association possible, it is the position of the authors that the proper scheme of analysis for hair evidence should start with a microscopical examination utilizing a good quality high-powered microscope followed by the appropriate type of DNA analysis.

Microscopical Hair Examinations and Limitations

Forensic microscopical hair examination and comparison is a discipline grounded in comparative biology, microscopy, anatomy, histology, and anthropology. In addition to the biological processes, subsequent chemical treatment of the hair and accidental changes or damage due to an individual's environment create extra dimensions useful for comparison.

The microscopical examination and comparison of hair is relatively straightforward and requires the use of one's hands and eyes, logic and reasoning skill, and high quality microscopes. Stereo, polarized, and comparison light microscopy are commonly used to conduct hair examinations and comparisons by appropriately trained practitioners. First, the gross morphology of the un-mounted hair is examined for its shape, color, curl, and length. Along with the racial attributes, the color of the hair may provide the most useful characteristic for investigative leads and for direct subsequent comparison testing. The perceived color of a hair depends on its pigmentation, transparency, and reflectivity. The surface of the hair is examined for trace materials or debris that might be present. This could also provide useful investigative information about the hair donor. As an example, a hair which has automotive spray paint particles adhering to it may suggest the donor works in an automotive body shop. After removing and preserving any hair contaminants for further analysis and comparison, a detailed examination and comparison of the internal features of the hair is conducted. This is facilitated by mounting the hair on a glass microscope slide in a liquid having a refractive index close to that of the hair itself. The hair is inspected microscopically using stereo and compound microscopes from 10 to 600 times magnification from the root to the tip. Root characteristics such as shape, degree of pigmentation, presence or absence of sheathing or other tissue may be indicative of the method of hair removal. Abnormalities in the root area or proximal end of the hair may provide valuable comparative data. The shaft of the hair is examined noting characteristics such as the diameter, cross-sectional shape, indication of damage or disease, pigment color and distribution, as well as the presence or absence of ovoid bodies or cortical fusi. The tip of the hair may be freshly cut, cut and worn (rounded), split or frayed, or angularly cut. At 200–600 times magnification, the outermost protective layer of overlapping scales (or cuticle) is inspected. The cuticle may be thick or thin, it can be colored by artificial treatment (dye) or display a natural hue. The scales may lay flat along the hair shaft or protrude to varying degrees. They can be broken, cracked, or even have a looped appearance. The inner cuticular margin (where the cuticle meets the cortex) can be clearly defined or have a gradual transition. This magnification also provides for the examination of the hair cortex with its keratinous fibrils and other various structures. The size, shape, color, and the amount and distribution of the pigment particles within the cortex are some of the most significant comparative features of hair. The presence or absence of cortical fusi (air spaces) and ovoid bodies (dark masses of pigment) as well as their morphology and distribution, can also be useful comparative features. The medulla, a channel at the center of the hair, may be absent or present, continuous or fragmented, opaque (filled with air), translucent (filled with fluid or proteinaceous material), or it may have a cellular appearance. The medulla can also vary between individuals in diameter and morphology. The hair examiner can also detect signs of damage, artificial treatment, or disease. Bleached or dyed hair is usually identified by a distinct demarcation between the treated and untreated portion of the hair shaft. The chemically treated hair shaft sometimes shows signs of damaged and separating cortical cells with an obliteration of the pigment particles.

This initial microscopical examination of a mounted hair can be completed by an appropriately trained hair examiner in a few minutes. Among other things, the racial characteristics, likely somatic origin of the hair (e.g., head, pubic, facial, body, or limb) and the suitability of the hair for microscopical comparison purposes can be ascertained during this examination. Generally, only head and pubic hairs possess sufficient microscopical characteristics for a microscopical hair comparison.

A microscopical hair comparison is made between a questioned hair and a known hair sample obtained from a victim, a suspect, or other relevant individuals. The recommendations for the collection of known hairs from subjects for comparison purposes is described in the Forensic Human Hair Examination Guidelines, Section 7, Forensic Science Communications, April 1, 2005. Each hair reference sample is studied in order to determine the range of colors, lengths, and other microscopic characteristics within the sample itself.

The questioned hairs are likewise characterized microscopically. Then, each questioned hair is compared to the reference hair samples using a high quality transmitted light comparison microscope. The side-by-side analysis is necessary in order to compare all of the subtle microscopic characteristics. In order to conclude that a questioned hair is associated or not associated with a known source, it must first be determined whether or not the characteristics exhibited by the questioned hair are within the range of characteristics present in the adequately representative known sample. If the questioned and reference samples demonstrate a similar range of like features, and the questioned hairs display no significant dissimilarities to the reference sample in the side-by-side comparison, the result is that the hairs are consistent to one another and it is concluded that the questioned hairs could have originated from the source of the reference sample. Conversely, if questioned hairs are microscopically dissimilar to the reference sample, then it is logical to conclude they are not consistent with originating from the source of the reference sample. Hairs that are similar in some features and different in others may result in an inconclusive opinion. When a hair is determined to be consistent to those from a particular individual, that hair should be evaluated to determine if it is suitable for mtDNA or nDNA analysis. When a hair examination results in an inconclusive opinion as to whether or not it originated from a particular individual, it should also be evaluated for potential mitochondrial or nuclear DNA analysis. A hair that is excluded as having come from an individual of interest by a microscopical examination should not require DNA analysis.

There are some limitations to microscopical hair examination. First and foremost, microscopical hair examination cannot conclusively determine if a questioned hair came from a particular source. It also does not provide information as to: the age or sex of the donor, time since the hair was shed, or the number of times a particular criminal act has occurred. Furthermore, many hairs are not suitable for comparison because of the size or type of hair (e.g., broken fragments, limb hairs, and body hairs). Incorrect associations can occur because it is possible for two individuals to exhibit similar microscopic characteristics in their hairs. Although this is unusual, it is more likely to be observed between hairs that have

relatively fewer microscopic characteristics, such as gray, light blonde, opaque, or very short hairs. This coincidental similarity in microscopic characteristics between the hairs of different individuals may lead to incorrect associations. Conversely, incorrect exclusions can occur when a questioned hair is "abnormal" in that it lies well outside the normal range of variation seen in a particular individual's hair. In these cases, the examiner may be correct in his/her assessment of the hairs, but the actual finding is incorrect. Incorrect associations and exclusions can also occur because of inadequate training, inadequate review, inadequate equipment, and the reliance on inadequate known samples. Incorrect findings of this type can be minimized through an adequate training program, a good quality assurance program, and insuring that the samples (both known and questioned) are suitable for comparison.

DNA Typing of Hairs and Limitations

Human hairs may be amenable to both nDNA and mtDNA typing. DNA analysis can often provide additional information to either include or exclude an individual as being the donor of a particular hair. A microscopical assessment of the hair for the presence or absence of a root, the growth phase of the root, the presence or absence of tissue attached to the root, and the presence or absence of other biological materials on the shaft of the hair will determine which mode of DNA typing should be employed.

With the exception of identical twins, nDNA analysis can potentially yield an association to a single individual. However, studies have shown that hairs from identical twins might be differentiated using microscopy. As powerful a tool as nDNA analysis is in establishing the potential identity or source of a hair, it does not provide other types of investigative information that might be an important aspect of a case. Establishing the presence of a victim's hair on a tool belonging to a spouse does not have the same meaning or significance as finding head hairs consistent to the victim's on the tool that exhibit damage consistent with crushing. In a similar situation, finding the victim's hairs in the trunk of the spouse's car wouldn't be unusual unless they displayed characteristic root banding which would indicate that they were lost from the victim's scalp some time after the victim's death. Finally, identifying a suspect's hair on the clothing of a sexual assault victim may not have the same impact as it would if that hair were identified as a pubic hair from the suspect. Unfortunately, most hairs found at crime scenes do not possess sufficient root tissue to get an nDNA profile. Hairs that possess roots in the anagen growth phase are actively growing and therefore still firmly attached to the follicle. The application of sufficient force to these hairs causes their removal and the roots of these pulled hairs are rich in mtDNA and nDNA. When hairs enter the resting phase (telogen), the root separates itself from the follicle, becomes bulbous, and is only held in place by the shape of the root. These hairs are shed very easily, and therefore may be deposited naturally throughout the day. For this reason, the majority of hairs found in casework do not have sufficient root tissue for nDNA analysis.

When no root tissue is present, mtDNA can often be extracted, even from very short hairs. mtDNA is maternally inherited, meaning it is passed on from the mother to her offspring. Therefore, all maternally related individuals are expected to share the same mtDNA profile. Furthermore, some unrelated individuals may exhibit the same mtDNA type. For this reason, it is particularly important to realize that the microscopical examination and mtDNA analysis are complementary techniques. In some instances, the microscopical hair comparison may be inconclusive because the hair is very short or fragmented or because a significant amount of time has passed between the commission of the crime and the collection of the known hair sample. These hairs can still often be analyzed for mtDNA. In other cases, where associating a questioned hair to one family member versus another, mtDNA may be less probative than a microscopical comparison. Microscopical comparisons can often result in associating a questioned hair to a particular family member versus another and in this situation the mtDNA may still be useful to confirm the putative association.

The primary limitation of both nDNA and mtDNA analysis is that they are destructive techniques because a portion of the hair must be digested to extract the DNA. Once this is attempted, the possibility of conducting a microscopical examination may be diminished or eliminated completely. Furthermore, useful profiles are not always obtained from a DNA analysis.

Case Examples

The microscopical examination and comparison of hairs has been upheld in Daubert hearings in courts in the United States. Expert testimony supporting hair examinations has been accepted in state and in federal courts throughout the United States and its territories. While there have been numerous cases in which expert testimony was provided, the following list of court cases includes those cases where the microscopical examination and comparison of hairs has been upheld in Daubert hearings and/or where the microscopical examination and comparison of hairs was significant to the case:

- State of North Carolina vs. Andre Jaren Edwards, 2001—Ginger Lynn Hayes and her 11-month-old son were abducted while making a stop at a CVS drug store in Greenville, North Carolina. Hours later, Ginger's body was found near her son who was alive but suffering from exposure. They were discovered in a field along with several abandoned tires. Numerous head hairs adhered to the rim of one of those tires were found to be microscopically consistent to the head hair sample from Ginger Hayes. The hairs on the tire had been crushed and broken, indicating the tire may have been used as the murder weapon. An autopsy revealed that blunt force trauma to the head resulted in Ms. Hayes' death. Testimony regarding the damage to the hairs was provided in federal court.
- United States vs. Anthony Zappa, 2002—Anne Sluti was kidnapped from a mall parking lot in Kearney, NE in 2001. She was taken through Wyoming and into Montana. Along the way, Ms. Sluti indicated that she was raped several times and was moved from place to place as the suspect fled from the authorities. Anthony Zappa was ultimately identified as the suspect. Hundreds of items from stolen vehicles and cabins were recovered and submitted as evidence, along with known hair samples from Ms. Sluti and Mr. Zappa. A large number of naturally shed head hairs (over 60) which were determined to be microscopically consistent to the victim's known head hair sample were recovered from two vehicles and three different cabins. This finding corroborated the victim's account of the situation. She indicated she was under duress and was not a willing participant as Tony Zappa had alluded to in his statements to the authorities. Mitochondrial DNA examinations were conducted on some of these hairs with the results supporting the microscopical hair examination conclusions. Testimony regarding microscopical hair examinations and mtDNA examinations was provided in Federal court. Zappa was convicted and sentenced to life in prison.
- State of Vermont vs. Alfred Brochu, 2004—This case involved the rape and homicide of Tara Stratton. The victim was the girlfriend of Alfred Brochu's son. Brochu claimed that he was at work on the night of the murder and that he could not have

killed her. Pubic hairs microscopically consistent with the known pubic hair sample from Brochu were found in the body bag that the victim was placed in and at the scene. Mitochondrial DNA examinations were conducted on these hairs and supported the microscopical examination results. A Daubert hearing challenging microscopical hair examination was conducted. The judge ruled that microscopical hair examinations are admissible. Testimony regarding the hair examinations and conclusions was provided as was testimony regarding the mtDNA examinations. Brochu was convicted on all charges.

- State of California vs. Scott Peterson, 2004—On December 24, 2002, Laci Peterson went missing from her home in Modesto, California. Her remains as well as those of her unborn son, Connor, were recovered from the San Francisco Bay in April 2003. In June 2003, two head hairs recovered from a pair of pliers identified as having been recovered from Scott Peterson's boat were submitted to the FBI Laboratory for mtDNA analysis. As per protocol, these hairs were first compared microscopically to the hairs recovered from a hair brush identified as belonging to Laci Peterson as well as a known head hair sample from Scott Peterson. The hairs from the pliers were found to be consistent with the hairs from the hair brush of Laci Peterson and were sent for mtDNA analysis. The mitochondrial DNA results supported the results of the microscopical examination of the hairs. In September 2004, testimony regarding the microscopical comparison of the hairs as well as the mitochondrial DNA analysis was provided. In November 2004, Scott Peterson was found guilty on all charges and was sentenced to death in December 2004.
- State of Florida vs. Joseph Smith, 2005—Eleven-year-old Carlie Brucia was abducted from outside a carwash in Sarasota, Florida in the early evening of February 1, 2004. Her body was found 4 days later in a church parking lot. The abduction was caught on a security camera at the car wash. The video was broadcast nationally and led to multiple tips eventually identifying Joseph Smith as the suspect. A vehicle that had been borrowed by Smith was located and processed for evidence. Several Caucasian head hairs were found on items from the vehicle that were microscopically consistent with originating from the victim. Additionally, multiple fiber associations were found between the victim's shirt and items recovered from the vehicle. In November 2005, testimony was provided regarding the hair and fiber evidence. Joseph Smith was convicted on all counts on November 17, 2005 and was sentenced to death. The death sentence was upheld by the judge in March 2006.
- State of Kansas vs. Marcy Carpezza, 2005; State of Kansas vs. Jason Hughes, 2005; State of Kansas vs. Gail Bennett Jr., 2005—These three cases resulted from the homicide of an elderly woman in Emporia, Kansas. A head hair was found under the victim's fingernail which was microscopically dissimilar to the known head hair sample from the victim. Head hair samples were submitted from 13 individuals and DNA samples from 18 individuals. The known head hairs from the suspects were examined and all were found to be microscopically dissimilar to the questioned hair. Because head hair samples could not be collected from all of the suspects, mtDNA examinations were conducted on this hair. The mtDNA profile of the questioned hair was consistent with that of the victim; however, the previous microscopical examination had eliminated the victim as the source of the hair under her fingernails. These findings prompted the investigators to look at maternal relatives of the victim as possible suspects. Further enquiries revealed that the victim's daughter was often at the victim's home. Requests for a known hair sample from the victim's daughter were denied; however, testimony was provided by both the hair examiner and the mtDNA examiner regarding these results.
- State of New York vs. Arial Menendez, 2006—Elizabeth Butler, a teenager, was allegedly raped and killed in her car at a train station. A pubic region hair was found on the victim's shirt which was microscopically similar to the pubic hair sample from Arial Menendez, her former boyfriend. Due to the limited nature of this hair, a stronger conclusion regarding the microscopical hair examination could not be made. A forcibly removed head hair which did not contain follicular tissue was found on the suspect's shirt. This hair was microscopically consistent with the known head hair sample from the victim. Both hairs were submitted for mtDNA examinations with those results supporting the microscopical hair conclusions. Testimony was provided by both the hair examiner and the mtDNA examiner regarding these results. Menendez was convicted of all charges.
- State of New York vs. Anne Trovato, 2006—Patricia Mery was found deceased after being stabbed multiple times and beaten with a bat. Cell phone records placed her estranged daughter, Anne Trovato, near the crime scene. Two head hairs on a knife found at the crime scene were compared to known head hair samples from the victim and her daughter. Like most hairs encountered in casework, the hairs from the knife had no apparent tissue and accordingly were only suitable for mtDNA analysis. Since Patricia Mery and Anne Trovato were maternally related, mtDNA results included both the subject and the victim as possible donors of the hairs. Prior microscopical examination of the hairs however, concluded that the hairs from the knife were not consistent with originating from the victim but that they could not be excluded as having originated from the suspect. Hair and mtDNA examiners testified in October, 2007 and Trovato was convicted.

Conclusion

The combination of microscopical hair comparison and nDNA or mtDNA analysis provides the criminal justice system with significantly more probative information than either technique does alone. Both microscopical comparisons and DNA analysis should always be considered in any case where hair evidence is important.

Appropriate training and competency are required in order to effectively conduct microscopical hair comparisons. Although the training necessary to become a qualified hair examiner is extensive, the primary concern of a forensic laboratory should be to provide the criminal justice system with complete and accurate information about the evidence. A competent microscopical evaluation of hair evidence before it is submitted for DNA analysis can limit the number of hairs that are analyzed by DNA, thereby reducing cost and analyst time while also providing the most useful information.

Forensic hair comparison can provide reliable and probative information in a criminal investigation. The type of information provided by a hair comparison is different but complementary to that of DNA analysis and therefore these examinations should not be eliminated. In a time when hair evidence has the potential to be a more powerful tool than ever before, it is imperative that it be analyzed with the best possible scheme of analysis.

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