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Little Variation Exists Between Laboratory Procedures for the Microscopical Examination of Human Hair

ABSTRACT

Microscopical forensic hair examination is a fast, cost-effective, and efficient way to observe morphology and discern species characteristics of hair evidence. However, it is considered subjective and lacking in error rates and reliable statistics to support microscopical hair examination results. A 2009 review by the National Academy of Sciences (NAS) identified weaknesses in the field and provided recommendations for the advancement of the hair analysis discipline. These recommendations included improving training and proficiency testing, enhancing uniformity by developing field-wide protocols, and reducing subjectivity. This study aimed to compare procedures and hair comparison metrics used between individual examiners and whole laboratories, as well as characterize the amount of training received and the overall casework experience of forensic hair examiners, following the release of the NAS report. Hair examiner members of the American Society of Trace Evidence Examiners (ASTEE, n=116) completed a 23-question survey using SurveyMonkey. Education and training were uniform; the majority of examiners received 3 or more months of training (83.8%) before performing independent analyses and had 5 or more years of experience (70.0%) as a hair examiner. Few examiners (8.1%) reported that laboratory procedures were affected by NAS report findings. Largely the same number and type of hair features are referenced by hair examiners during microscopical hair comparisons, indicating that little variability exists between full hair comparisons performed at different laboratories or between individual examiners, despite the lack of mandated, field-wide procedures.

Keywords: Trace Evidence, Hair Analysis, Microscopical Hair Examination

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INTRODUCTION

Human hair can exhibit a variety of distinguishing characteristics, and combined with its natural propensity to be shed, hair is a common piece of forensic trace evidence [1,2]. Microscopy is performed on hair evidence to confirm that a sample is indeed human hair, and to find similarities or discrepancies between the evidence and known hair collected for forensic analysis. Because of its usefulness, versatility, cost-effectiveness, and rapidity, microscopical analysis and comparison procedures using hair morphology have largely remained unchanged since the early 20th century [2,3].

Despite the benefits of microscopic hair examination, it is considered subjective and lacking in error rates and reliable statistics to support examination results. Two hair examiners may characterize the same hair differently based on the amalgamation of training, experience, and education they receive, and variation may exist within analyses by the same examiner [4,5]. Many analysts agree that hair evidence cannot be used to conclude with certainty that an unknown hair came from a particular individual, and that supporting analyses, including DNA testing, are required to make a more concrete conclusion [3,6–8]. No laboratory studies have outlined a successful process for the creation of unbiased, accurate probability studies or error rates that are recognized in the microscopical hair analysis field.

In 2009, the National Academy of Sciences (NAS) released a report outlining the status and future directions of forensic science and its sub-disciplines at the request of the United States Congress [9]. Hair examination practices were assessed, along with associated quality control methods, the accuracy and reliability of hair examinations, and scientific value they provide. Hair examination was determined to be one of the most vulnerable disciplines in forensic science. The report interpreted that no standards or protocols exist outlining how many hair features different samples need to have in common to be considered similar, or how much training is required for an analyst to be considered a competent, skilled hair examiner [9]. Due to the perceived lack of stringent, uniform procedures, error rates, and statistics supporting hair comparison results, the NAS concluded that microscopical hair comparisons should only be performed in conjunction with mitochondrial DNA (mtDNA) analysis, and have no utility on their own for individualization purposes, although the report noted that further investigation should ascertain whether any quantifiable benefit exists when combining microscopical examination results with mtDNA testing [9].

The purpose of this study was to identify the practices and procedures being followed in the field of hair examination and comparison following the request of a large-scale procedural overhaul by the NAS. Determining how comparisons were being performed would elucidate the purported variation between hair comparison methods, as well as highlight whether the issuance of the NAS report has unified the actions of hair examiners. Variability in training and work experience of analysts, as well as their views regarding the value of hair comparison evidence, were also assessed.

MATERIALS AND METHODS

A 23-question online survey was created using SurveyMonkey (www.surveymonkey.com, Palo Alto, CA) and a link was distributed electronically to forensic trace evidence examiners within the American Society for Trace Evidence Examiners (ASTEE) between October and December 2012 (Appendix 1). General questions appropriate for any trace evidence examiner were used to gauge which laboratories were represented and performed microscopical hair analysis. If the ASTEE member did not perform microscopical hair examinations, the survey ended following the general question portion. If the ASTEE member affirmed that he or she performed any type of microscopical hair examination, the survey continued with specific questions regarding laboratory experience, training, and test methods. Those who perform full microscopical hair comparisons between known and unknown samples were asked to indicate which of 26 specific hair features were used in their comparisons. These features were adapted from guidelines published by the Scientific Working Group on Materials Analysis (SWGMA) [10]. Those surveyed were asked the name of their laboratory of employment to ensure no data duplication occurred.

Survey data was analyzed by individual examiner and by laboratory. Data from multiple examiners from the same laboratory were consolidated using means to create a representative sample from that laboratory; no differences existed between representative laboratory data and individual examiner data. Discrepancies in data between examiners within the same laboratory were addressed by selecting the data reported by the examiner with the greatest length of experience and training or where supplemental rationale was provided. Only when an ASTEE member performed full microscopical hair comparisons was his or her list of specific hair features included in the analysis. The total number of features each laboratory and individual used during comparisons was summed (e.g. 23 out of 26 possible features). Data is reported as a percent or mean \pm standard deviation (SD) as indicated.

RESULTS AND DISCUSSION

Survey data was collected from 117 participating members of the ASTEE from 66 laboratories. Of the laboratories represented, 89.4% were accredited by the American Society of Crime Laboratory Directors- Laboratory Accreditation Board (ASCLD-LAB) or Forensic Quality Services (FQS). One member surveyed performed animal hair examination only, and was excluded. Nine members surveyed did not include a laboratory of employment and were excluded from questions regarding laboratory practices as a whole to avoid unintentional data duplication. Data reflecting the geography of participating laboratories is listed in Table 1.

Table 1: Geographic region of responders.

Region	By analyst (n = 116)		By lab (n = 66)	
	N	%	N	%
New England	3	2.6	3	4.5
Mid-Atlantic	15	12.9	9	13.6
Midwest	28	24.1	16	24.2
South	24	20.7	13	19.7
Southwest	10	8.6	6	9.1
West	25	21.6	17	25.8
International	2	1.7	2	3
Unknown	9	7.8	N/A	0

Eighty-five survey respondents from 57 different laboratories perform hair examinations, comprising 77.3% of surveyed participants (Figure 1). A majority of hair analysts have performed hair examinations for five or more years (70%) and were trained for three or more months (83.8%; Figures 2 and 3). Out of 26 hair features identified in this survey as comprising a full hair comparison, 24 ± 2 features were used by each individual analyst and each laboratory. Specific information regarding the frequency of use of each hair feature by analyst and by laboratory is depicted in Table 2. When asked whether the NAS report affected the performance of hair analyses in each laboratory, 87.1% of laboratories reported that it had not.

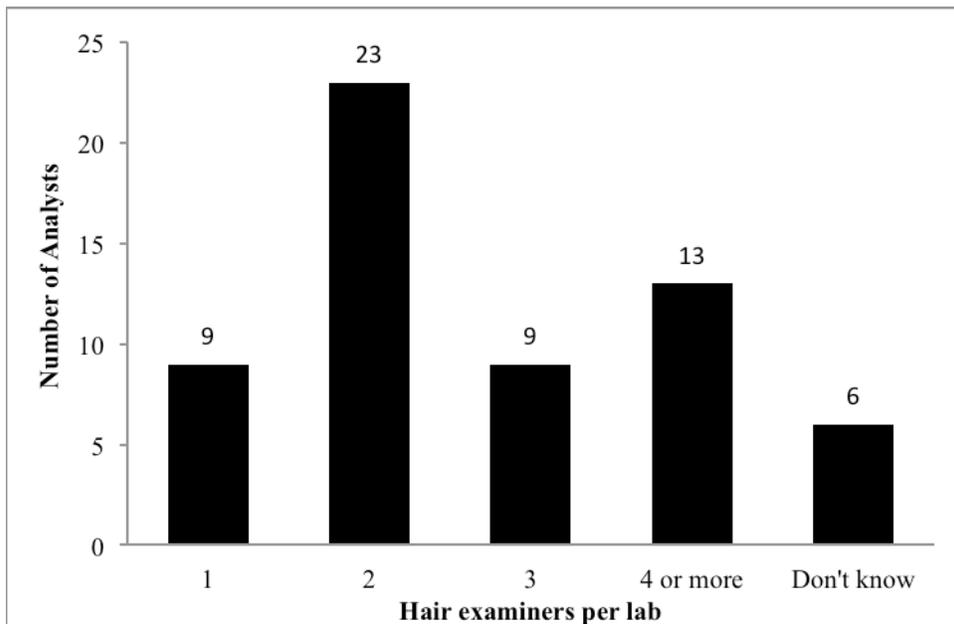


Figure 1: Number of hair examiners employed per laboratory surveyed

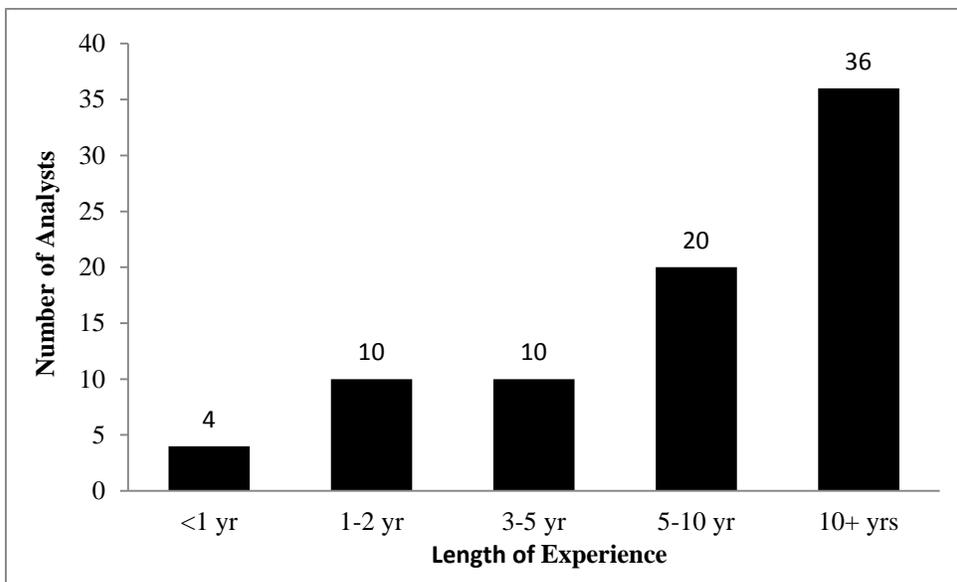


Figure 2: Length of professional experience performing hair examinations of surveyed analysts

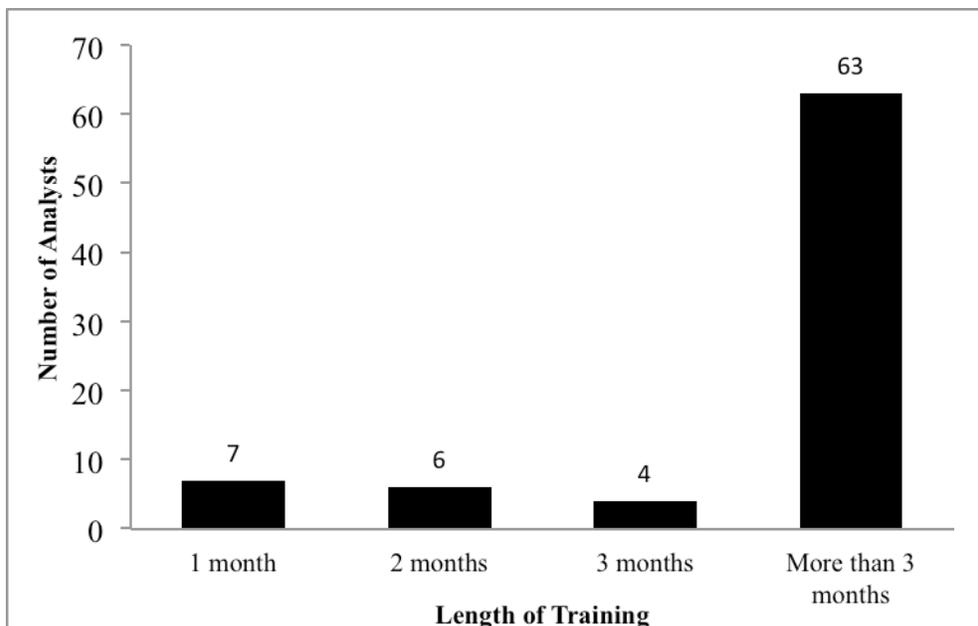


Figure 3: Length of hair examination training received before independent analysis was permitted

Table 2: Hair characteristics examined during microscopical hair comparison.

Characteristic	Used by analyst (%)	Used by lab (%)
<i>Origin</i>		
Human/animal origin	100	100
Body origin	98.5	97.4
Racial origin	81.8	76.9
Root identification	98.5	97.4
<i>Shape</i>		
Form	100	100
Shaft	100	100
Proximal ends	97.0	100
Distal ends	100	100
<i>Color and pigment</i>		
Hue	100	100
Color intensity	98.5	100
Artificial treatment	100	100
Pigment size	95.5	92.3
Pigment density	97.0	97.4
Pigment distribution	97.0	97.4
Pigment aggregation	100	100
Pigment aggregate size	90.0	94.9
<i>Cuticle, Medulla, and Cortex</i>		
Outer cuticle margin	86.4	92.3
Inner cuticle margin	74.2	79.5
Cuticle thickness	100	100
Cuticle appearance	69.7	69.2
Medulla	100	100
Cortex	100	97.4
<i>Acquired characteristics</i>		
Disease states	78.8	79.5
Non-color treatment	60.6	56.4
Damage	97.0	97.4
Artifacts	92.4	97.4

Preliminary screening for DNA purposes was done by 93.6% of analysts, while 82.5% reported performing full microscopic hair comparisons (Figure 4). Microscopical hair comparisons precede analyses for both nuclear DNA (nDNA) and mtDNA in a majority of laboratories (Figure 5). The opinions of 64 hair analysts regarding the utility of hair examinations are represented in Figure 6. Most commonly, hair examinations are viewed as supplementary evidence to be used in conjunction with DNA testing, as reported by 59.4% of analysts. Hair evidence was viewed as suitable standalone evidence without accompanying DNA data by 18.8% of analysts, and as a screening tool only by 7.8% of

analysts. Nine analysts reported that none of these definitions represented their view of hair examinations and provided comments to explain their responses.

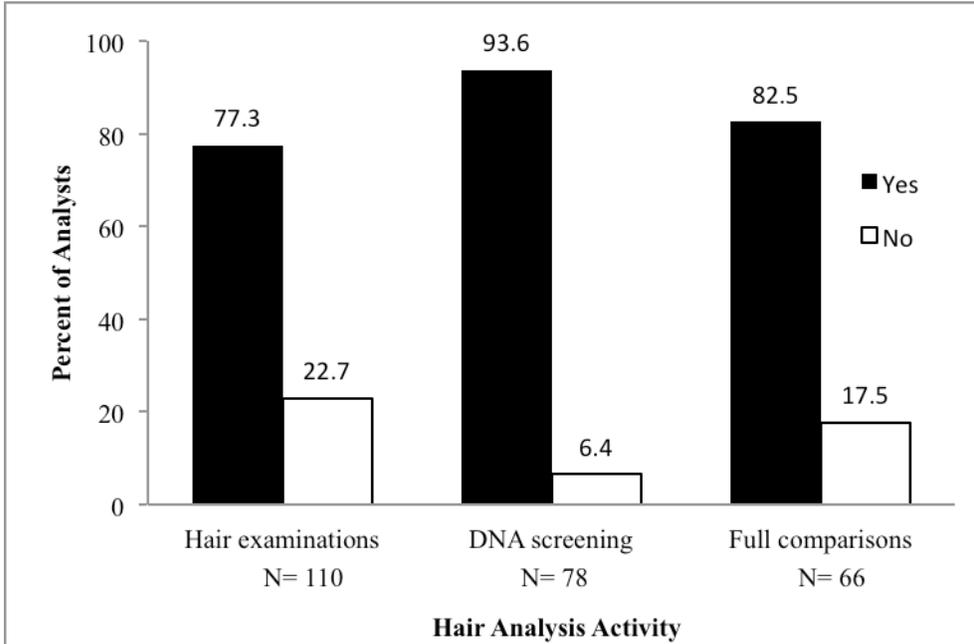


Figure 4: Percentage of analysts who perform hair analysis activities including examinations, DNA screenings, and full comparisons

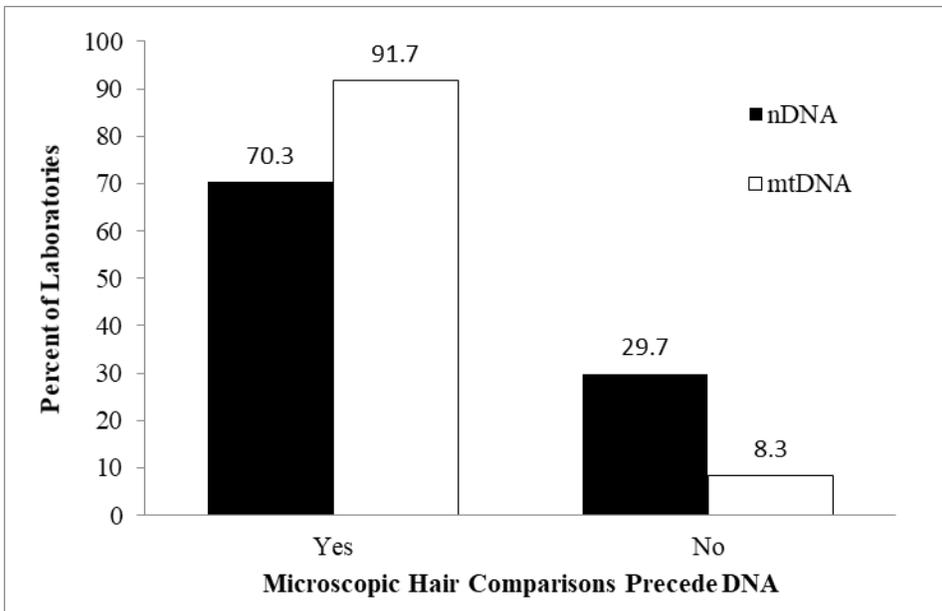


Figure 5: Percentage of laboratories reporting whether microscopical hair comparisons precede nDNA and mtDNA analysis

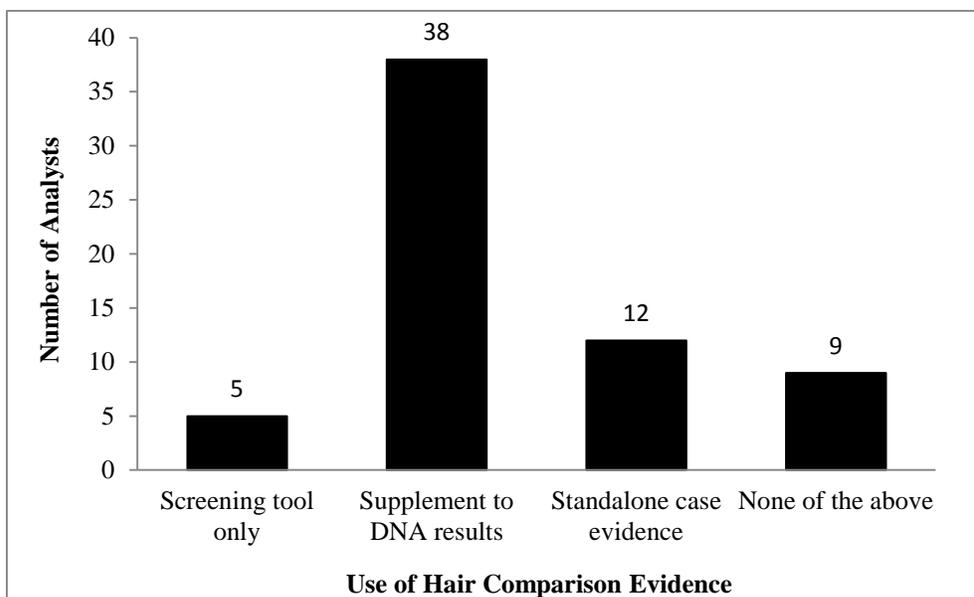


Figure 6: Current opinions of hair analysts regarding the use of hair comparison evidence

Procedures and NAS Impact

The NAS report recommended there be uniform hair examination procedures established and maintained in each laboratory. Little variation existed between the microscopical hair comparison procedures of each analyst or each laboratory. Of the hair features identified by SWGMAT, laboratories and individual analysts referenced nearly all of them during hair comparisons, with eight of 26 features being used by 100% of analysts surveyed. Three of the features utilized least often were still referenced by over half of individual analysts and laboratories, demonstrating the uniformity of comparison metrics used. Some analysts contributed additional hair features they look for and tests they perform, suggesting that the SWGMAT list of features could be supplemented, potentially providing further discrimination between samples.

Additionally, very few examiners reported that any laboratory procedures had been affected by the issuance of the NAS report. In laboratories where the NAS report had influenced procedures, specific report wording and clearer statements of the limitations of hair examinations were added to all forms of documentation. The NAS report led to the institution of a secondary examiner review policy following hair comparisons in one surveyed laboratory, and a stark shift to use of hair screenings as a suitability precursor to DNA analysis only in another.

Education and Training

The NAS report urged laboratories to ensure that regular proficiency testing and thorough, proper training was being performed in each laboratory. A large majority of laboratories surveyed were accredited, indicating that high standards of proficiency testing are met in most hair analysts. Most hair examiners received training that lasted three or more

months before independently assessing evidence, and a majority have between 5–10 years, and in many cases, 10 or more years of experience. The training regimen did not appear to be waning in new employees, as analysts with less than one year of hair examination experience also received over 3 months of training.

Prevalence and Significance

Conclusions from the NAS report indicated that forensic hair analyses do not provide valuable results without being directly accompanied by DNA data. Despite the perceived lack of significance, hair examination is still quite common, as some form of hair analysis, whether it be DNA suitability or full hair comparison, was being performed at almost all of the laboratories that participated in this study. Hair examinations were performed in the trace section of laboratories according to 87.6% of analysts, but the remaining analysts explained that hair examination had been omitted from the trace section in favor of the biology or DNA section, or from the laboratory entirely. This trend coincided with the finding that hair analysis was listed as part of the biology or DNA sections as opposed to trace evidence sections in one in five laboratories. One analyst was part of a large hair examination program comprised of several proficient analysts when the program was discontinued in favor of suitability analysis in the DNA section. Respondents reporting reallocation of hair examinations to the DNA section stated this was due to discontinued proficiency testing and training, changes in technology, and management decisions regarding resource use. However, since an overwhelming majority of laboratories surveyed performed microscopical hair examinations within the trace evidence section, this trend was not found to be pervasive within the field.

Perceived value of microscopical hair analysis

Hair analysts provided several strongly-worded justifications and beliefs regarding the value and utility of hair examinations, which demonstrated their will and passion for processing hair evidence. When asked how hair examinations should ultimately be used in the forensic field, many analysts were hesitant to commit to just one answer. A majority felt that hair examinations should be used in conjunction with DNA testing, while smaller groups of analysts considered it to be an acceptable form of standalone evidence or a screening tool only. The remaining analysts instead remarked that the utility of hair examinations was multi-faceted, and wished they could have selected multiple answers depending on the circumstances of the case. Additionally, analysts expressed that sample quality, morphology, and the presence of complimentary mtDNA or nDNA evidence in the case would also impact how the evidence best be used.

Despite the recommendation of the NAS report that hair evidence be used exclusively when accompanied by DNA test results in court, several analysts believed that a hair comparison is more valuable than the NAS report implies. Although hair examinations were often considered supplemental to DNA testing by analysts, they were not viewed as a sufficient replacement for DNA testing. Many analysts listed benefits of hair examination

that DNA testing simply could not provide, to include ascertaining information about the crime scene in the case of burnt hair, circumstances of the crime itself in the case of forcibly removed or decomposing hair, or morphological discrimination between victims or suspects having the same mtDNA profile. If DNA analysis is ultimately unsuccessful due to insufficient length or root viability, data from a previously performed hair examination may still provide racial origin information or body origin of the hair, which may add value to the case. Additionally, DNA testing is often time consuming and costly, so if a hair sample is at the bottom of an evidence backlog, or financial constraints are preventing a timely examination of case evidence, a microscopical hair examination may serve as a fast and inexpensive method of gaining information and making exclusions in the case.

Disagreement existed among analysts as to whether hair examinations and comparisons could stand alone in court. The most important factor regarding the clarity and effectiveness of hair examination results was that they be reported with the appropriate level of significance. Several analysts who believed that hair examination conclusions may be used as standalone evidence explained that as long as the reports and testimony reflect the specific meaning and limitations of the testing, the hair could be presented as exclusionary evidence. A conservative approach to inclusions should still be observed, as analysts agreed that it is more appropriate when in doubt to keep a number of possible exclusions rather than wrongly include any samples. Several analysts expressed the concern that hair evidence was being represented improperly in court, the fault for which could be attributed to the analyst for not clearly explaining the weight of the evidence, or the attorneys for drawing the wrong conclusions themselves. Uniform, standardized language being developed currently by the Department of Justice [11] and Organization of Scientific Area Committees- Chemistry/Instrumental Analysis Materials (Trace) committee [10], could potentially reduce confusion when an analyst is ambiguous about the significance of evidence or the attorney is unclear of its implications, but analysts opined that it was of utmost importance that a hair examiner explain that a hair comparison alone cannot lead to positive identifications in court.

Hair analysts were aware of the stigma the NAS report has imparted and the trend toward reduction of microscopical hair comparisons in favor of root screenings for DNA viability, but did not agree with it. Some analysts believed that confusion and overstated significance of hair comparison evidence before DNA technology was available, or when DNA evidence was not present in a case, has led to false convictions which are presently being exposed by DNA testing and have tainted the reputation of hair analysis as an invalid science. Based on the comments provided in the survey, hair analysts seemed receptive to recommendations by the NAS report, to include the use of consistent terminology in reports, uniform protocols, rigorous training, and frequent proficiency testing, and would likely strive to improve in all areas to increase respect for the hair examination discipline. Hair analysts appeared to crave the direction and rigidity of a

concrete, stable set of methods and standards with which to perform their work with maximal legitimacy.

CONCLUSIONS

Forensic hair analysis methods, uses, and comparison procedures were identified and assessed for variability in hair examiners. Despite criticism of hair analysis in the NAS report, hair examinations and comparisons remained commonly practiced. A large majority of analysts received the same amount of training and had many years of experience in the field; however, very few reported that their laboratory procedures were affected as a result of the NAS report. The same number and type of hair features were being referenced when microscopical hair comparisons are performed. A trend toward the performance of more frequent root screenings for DNA viability and less frequent full hair comparisons was detected and supported by a shift of hair analysis from trace sections to biology or DNA sections. Hair examiners believed that, despite any stigma inferred from the NAS report, hair examinations and comparisons provided valuable contributions to a forensic case.

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DISCLAIMER

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or the Department of Defense.

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Appendix 1. Microscopic hair examination survey

1. What is the name of the lab where you work? (This information will only be used for database duplication purposes, it will not be published in the findings)

Text box

2. Do members of your lab perform any type of microscopic hair examinations (i.e. preliminary screenings or full hair microscopic comparisons between known and unknown hair samples)?

Yes (if yes, skip to Q4) / No (if no, skip to Q3) /
I don't know (if I don't know, skip to Q4)

3. Has your lab performed microscopic hair examinations in the past, but stopped performing them currently?

Yes / No / I don't know
If yes, please state the reason, if you know it:

4. Does hair examination fall under the Trace Evidence discipline in your lab?

Yes / No / I don't know
If no, please state the discipline under which it falls:

5. How many hair examiners are there in your lab?

1 / 2 / 3 / 4 or more / I don't know

6. Did the information found in the NAS report of 2009 affect how hair examination is performed in your lab?

Yes / No / I don't know
If yes, please describe how it affected your hair examinations:

7. Do you personally perform microscopic hair examinations?

Yes / No (if no, survey ends here)

8. How long have you been performing microscopic hair examinations?

Less than one year
1-2 years / 3-5 years / 5-10 years / 10+ years

9. How long did you receive training in microscopic hair analysis before you began performing your analyses independently?

1 month / 2 months / 3 months / More than 3 months

10. Do you perform preliminary hair screening for DNA purposes?

Yes / No

11. Do you perform full microscopic hair comparisons with known and unknown hair samples?

Yes / No

12. Regarding basic hair features, which of the following microscopic identifications do you perform? Check all that apply:

Human/animal origin

Body origin (hair, pubic, etc.)

Racial identification

Root identification for nuclear or mitochondrial DNA testing

13. Regarding hair coloring and pigmentation, which of the following facets of hair do you use for comparisons? Check all that apply:

Artificial color treatments (dyed, bleached, etc.)

Color intensity (light, medium, opaque, etc.)

Hue (blonde, black, etc.)

Pigment size (coarse, fine, medium)

Pigment density (absent, light, medium, etc.)

Pigment distribution (uniform, peripheral, one-sided, etc.)

Pigment aggregation (streaked, clumped, etc.)

Pigment aggregate size (small, medium, large)

14. Regarding hair shape and condition, which of the following facets of hair do you use for comparisons? Check all that apply:

Form (straight, wavy, curly, etc.)

Shaft (buckling, splitting, regular, etc.)

Proximal ends (if root is present: growth phase, banding, etc.; if absent: severed, decomposed, etc.)

Distal ends (tapered, rounded, square, etc.)

15. Regarding further analysis of the hair root, which of the following features do you use to determine whether a hair is suitable for nuclear DNA analysis? Check all that apply:

- Anagen root
- Catagen root with sufficient germinal nipple
- Telogen root with sufficient germinal nipple
- Any visible tissue present

16. Regarding features of the cuticle, medulla, and cortex, which of the following facets do you use for comparisons? Check all that apply:

- Outer cuticle margin (smooth, cracked, etc.)
- Inner cuticle margin (distinct, indistinct)
- Cuticle thickness (thin, medium, thick)
- Cuticle appearance (clear, milky, etc.)
- Medulla (absent, continuous, discontinuous, etc.)
- Cortex (ovoid bodies, cortical fusi, size and distribution of each)

17. Which of the following acquired hair characteristics do you look for if necessary? Check all that apply:

- Disease states (pili annulati, monilethrix, etc.)
- Non-color treatments (hair products, perm/straightening treatments, etc.)
- Damage (burned, crushed, environmentally/chemically damaged)
- Artifacts (lice, mold, blood, etc.)

18. Please list any other additional forms of microscopic analyses you perform that have not been previously described.

Text box

19. If nuclear DNA is performed on the unknown hair(s), is a microscopic hair comparison performed prior to submitting the evidence to DNA?

Yes / No

If no, please state the reason:

20. If nuclear DNA is performed on the unknown hair(s), who performs this testing? Check all that apply:

- Nuclear DNA testing is performed in our lab
- Nuclear DNA testing is performed at an outside lab

Nuclear DNA testing is performed, but I don't know where
Nuclear DNA testing is not performed

21. If mitochondrial DNA is performed on the unknown hair(s), is a microscopic hair comparison performed prior to submitting the evidence to DNA?

Yes / No

If no, please state the reason:

22. If mitochondrial DNA is performed on the unknown hair(s), who performs this testing? Check all that apply:

Mitochondrial DNA testing is performed in our lab

Mitochondrial DNA testing is performed at an outside lab

Mitochondrial DNA testing is performed, but I don't know where

Mitochondrial DNA testing is not performed

23. How do you feel microscopic hair examinations should be used in the forensic field?

As a screening tool only

As supplementary evidence to the DNA results

As standalone case evidence, without the DNA results

None of the above

Please explain further, any of your answers:

24. If you have any further comments, please include them below:

Text box