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TECHNICAL NOTE

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A Comparison Study of Hair Examination Methodologies

ABSTRACT: A study was conducted to investigate the accuracy between two methods of hair analysis: PCR-STR DNA analysis and microscopic comparison analysis. Standard sets of pubic hairs were collected from volunteers, and unknown sets were generated from these samples. Three out of five (60%) of the hairs analyzed produced full DNA profiles that were correctly matched to the standard sets. DNA analysis was inconclusive (partial or no DNA profile) for two out of five (40%) of the samples. In contrast, the microscopic comparison analysis correctly matched four out of five (80%) of the samples to the standard sets but mis-identified one out of five (20%) of the samples. These results reinforce the practice of preliminary microscopic hair examination in narrowing down a set of hairs for DNA analysis. Microscopic comparison analysis is sufficiently reliable to remain a rapid and inexpensive method for forensic hair analysis.

KEYWORDS: forensic science, hair analysis, microscopic comparison, DNA typing

Hair, because of its ubiquity at crime scenes, has great evidentiary value in forensic science. Hair was first used as evidence in 1861, but did not gain scientific acceptance until after the turn of the century, and public acceptance until the late 1950s (1). When testifying in court, forensic scientists are mandated to use terminology such as "consistent" and "similar to" when reporting on the origin of hairs. Because of the subjective nature of the analysis, hair is not usually the sole evidence in a trial, and hence "hair comparison evidence is generally only of value when used in conjunction with other evidence" (2). As evidence, hair can tell an experienced analyst much about the possible source, and with the typing of mitochondrial or nuclear DNA, the forensic profile of an individual. While this information is useful and necessary in the practice of solving crimes, hairs are limited in that, when analyzed using traditional microscopic methods, they cannot positively identify an individual.

Although great strides have been made in the field of traditional hair examination, several challenges remain. In essence, the examiner must find a hair which differs from all other hairs in a setting. The characteristics of this hair can be used to separate a suspect's hair from the hairs of the victim or resident. This question of separation and identification is always the key issue with any biological evidence, and much of the success in this endeavor has been attributed to the use of DNA technology. DNA typing has a proven ability to identify/individualize samples and to provide a mathematical statement regarding the strength/significance of an association. Thus, DNA technology is capable of providing a level of individualization toward unknown hairs; a level that cannot be achieved simply with microscopic comparison. However, microscopic hair analysis is rapid and inexpensive and could be used to screen the abundant and ubiquitous hair evidence prior to the more time consuming and expensive DNA testing.

The research described herein used standard sets of pubic hairs to compare the accuracy between microscopic hair analysis and DNA analysis.

Methods

Pubic hairs were collected from 27 volunteers employed in the Department of Forensic Biology at the Office of Chief Medical Examiner in New York City (OCME-NYC). All volunteers agreed to participate by signing an informed consent document approved by the Internal Review Boards at the John Jay College of Criminal Justice, CUNY, and the New York City Department of Health. The laboratory staff was an ideal choice as research subjects, due to the mandatory quality assurance/control program in the laboratory. In accordance with ASCLD/LAB guidelines, and to establish a system of internal controls, each staff member's DNA profile has been generated with all of the commonly used STR systems in the department, and a database containing this information is available. This database was searched against the generated profiles from the "unknown" hairs donated by the volunteers.

Each volunteer was issued a packet that contained collection instructions, a medium sized coin envelope coded with a five-digit random number, a comb, and a strip of evidence tape. Each subject was to provide a minimum of 50 pubic hairs collected in a manner which maximized the number of anagenic/catagenic hairs in the standard sets. The volunteers were asked to follow a specific

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collection procedure that involved a thorough combing of the pubic region to remove any possible extraneous and non-personal hairs. Pubic hairs were then collected by plucking or massaging the hairs out of the skin from the entire pubic region. The collection procedure continued as many days (one to seven) as necessary for the subject to collect a minimum of 50 pubic hairs. The pubic hairs were collected and returned in the coded coin envelope. An impartial third party examined the standard sets and created five sets of four hairs each to act as unknowns. The group of four hairs that made up one unknown set was from the same sample and two out of the four hairs had to contain root sheath material (anagenic hairs). The coded envelopes and the five unknown sets were then transferred to the principal investigator.

The five sets of newly created unknown hairs were examined set by set, and two anagenic hairs were retained from the four unknown hairs per set. One of the two retained hairs from each unknown was cut to isolate the root end cells, and the DNA was extracted using the Chelex (3) extraction protocol as validated and defined by the Department of Forensic Biology (personal communication, Dr. Robert Shaler, OCME). A positive control hair from the principal investigator was extracted along with the unknown set.

The amount of DNA present from each extraction was determined using slot blot quantitation (4), and the extracted DNA was amplified using the AmpFℓSTR COfiler (PE Applied Biosystems, Division of Perkin Elmer, Foster City, CA) amplification system (5). Microcon-100 (Amison, Division of Millipore, Bedford, MA) purification and concentration were performed on any samples that gave negative quantitation results. The Cofiler DNA amplification system targets six STR loci plus the gender determining amelogenin locus. The STR profile was obtained using ABI Prism 310 (PE Applied Biosystems, Division of Perkin Elmer, Foster City, CA) capillary electrophoresis and Genescan genotyper software, Version 2.5.

The entire DNA analysis was duplicated using the second of the two hairs from the unknown groups. The results of the two runs were compared and used to generate a DNA profile. This DNA profile was then compared to the laboratory staff DNA database, and if a matching profile was found, the name of the laboratory member was recorded as the result of the STR DNA comparison. If a partial type was present, all names in the database that contained that partial profile were recorded, and the standard set numbers for each name were reported. As profiles were generated for the unknowns the statistical probability for each profile was determined using a macro spreadsheet based on Bayesian population calculations.

The remaining hairs in the 27 standard sets, the two leftover shafts and the two remaining hairs in each of the five coded unknown sets were examined and compared microscopically. Analyses were performed according to SWGMAT guidelines. Five representative hairs from each of the 27 standard sets were mounted and catalogued. The hairs from the five sets of coded unknown hairs were mounted, examined, and compared to the catalogued standard sets in order to determine the origin of each unknown hair.

Results from the hair analyses were then compared to the DNA results, and similarities and differences were noted. The DNA results and the hair analysis results were then compared to the master codebook to determine concordance between reported and known sample origins.

Results and Discussion

The results of the STR DNA and microscopic analyses are shown in Table 1. Although the sample size is small and further research is needed, the findings have important implications for forensic casework.

TABLE 1—*Individualization of pubic hairs using STR DNA and microscopic hair analyses.*

Set	DNA Results	True Unknown	Hair Analysis Results
	2 hairs		4 hairs
1	#26430	#26430	#48951
2	INC	#60445	#60445
3	#61679 or #63290	#63290	#63290
4	#75385	#75385	#75385
5	#88063	#88063	#88063

INC: inconclusive.

STR DNA typing of the unknown hairs correctly individualized hairs from sets #1, #4 and #5. The first replicate of set #2 and set #3 produced negative quantitation results. These samples were concentrated (microcon) and again, the quantitation results were negative. In casework, samples yielding a negative quantitation value would not be analyzed further, but for this research, an attempt was made to amplify both the original extract and the concentrated sample to determine if any alleles could be detected. All of the negative samples were amplified using 20 μL of the extract or concentrated sample.

The DNA analysis of the hairs in unknown set #3 yielded a partial profile, namely a heterozygous type at the D3S1358 locus and a homozygous X at the Amelogenin locus (indicating a female). Based on the search of the Labstaff database, only two possible female donors could have contributed this unknown hair. Further individualization could not be performed with such limited DNA STR data. Ultimately, it was determined that the donor was one of the two possible females identified in the Labstaff database search. The hairs in unknown set #2 could not be individualized due to a complete absence of STR data. Overall, the DNA STR analysis individualized three of the five unknown hairs (60%). Since there was a 50% chance of correctly matching the hairs in unknown set #3 (with the partial DNA profile) to the donor and each set contributed 20% toward the total success rate, an additional 10% could be added to the overall success rate for the DNA analysis. If the result for unknown set #3 is counted toward the total success rate, then the overall success rate for the STR DNA is 3.5 out of 5 (70%).

The microscopic hair comparison analysis correctly matched hairs in the unknown sets #2, #3, #4, and #5 to the standard sets from which they were generated. The hairs in unknown set #1 were not correctly matched to the donor standard set. Thus, microscopic hair analysis had a success rate of four out of five (80%) for matching the unknown hairs to the donor standard sets.

In contrast to the STR DNA analysis, microscopic hair comparison exhibited a one in five (20%) error rate. The unknown hairs in set #1 were correctly matched to the original standard set by DNA STR analysis, but were incorrectly matched to the donor set by the microscopic hair analysis. It must be noted, however, that these hairs would not normally have been analyzed in casework due to the small size of the hair.

The hairs in unknown set #2 were not matched to the original standard set by DNA STR analysis, but were correctly matched to the donor set by microscopic hair comparison. In this instance the results from the microscopic hair analysis were more informative than those from the DNA analysis.

The hairs in unknown set #3 were partially matched to the original standard set by DNA STR analysis. However, based on this partial profile, a second female individual could have been the source of

these hairs. In contrast, the unknown hairs in set #3 were correctly matched to the standard set by the microscopic hair comparison. Therefore, despite the fact that the DNA STR analysis had correctly narrowed down the sources (two females one of whom was the donor) of the unknown hairs, the microscopic hair comparison matched the unknown hairs in set #3 to the standard set.

The hairs in unknown sets #4 and #5 were individualized to the donor standard sets by both the DNA STR and microscopic analyses.

The second set of unknown hairs was extracted to attempt to duplicate the results of the first set. The results duplicated those obtained in the first analysis.

The main problem encountered in performing DNA STR analysis in this study was the limiting amount of the cellular material present in the root sheaths of the unknown hairs. In all types of DNA casework, the amount of DNA present in a sample is the determining factor in the overall usefulness of a sample. Typically in forensic casework, the ideal types of samples are bloodstains, semen stains, saliva stains, and orifice swabs. These samples provide roughly constant amounts of genomic DNA that can be successfully typed during standard STR casework. Non-ideal samples like hair do not provide consistent amounts of DNA and are, therefore, not primarily used in forensic casework. Because of the inherent difficultly of sorting through hairs, finding an intact root structure, and then successfully extracting DNA, hairs can sometimes become a "last resort" in forensic DNA investigations.

There is a possibility that with different STR amplification systems, such as Profiler + or Powerplex, the low-yield samples could have generated more data because of the larger number of loci and higher sensitivity in these systems compared to the Cofiler system. The Cofiler amplification system is less sensitive than either of the two previously mentioned systems, but because of the limited scope of the study and the small sample set, statistics in the one-in-a-million range were more than sufficient, and this was easily achieved with a full 6-locus profile from Cofiler.

This study has shown that these two independent systems of hair analysis have their unique place in the field of forensic hair analysis. Independently, both systems have been shown to be successful in identifying/individualizing unknown hairs. The microscopic hair analysis was more successful due to the presence of a large number of microscopic minutiae within a hair and the experience of the examiner. STR DNA was less successful due to the lack of sufficient quantities of DNA in some of the hair roots. As previously suggested by Linch et al. (6), and supported by Houck and Budowle's (7) study comparing microscopic hair analysis and mitochondrial DNA analysis, the most useful information would be obtained by using both of these two systems in tandem-microscopy first to narrow down the scope of the investigation, followed by DNA analysis to individualize the separated hairs. Similarly, the FBI SWGMAT committee strongly urged that the results of any hair analysis should be verified with DNA analysis (personal communication, Nicholas Petraco, NYPD Forensic Laboratory). In the suggested methodology for microscopic hair analysis, the committee focused on the secondary use of DNA technology, citing that "inconclusive results, at the comparison microscopy level are likely to be resolved by DNA profiling."

Microscopically, the hairs from a single investigation can be rapidly compared against the standard sets and questioned hairs can be quickly identified, especially when starting with a large sample set. This rapid, low-cost preliminary separation would be followed by the more time consuming and expensive DNA analysis of the questioned hairs.

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