



### **B177 Microscopic Groupings of Hairs as a Basis of Sample Selection for DNA Testing: Feasibility and Examples**

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After attending this presentation, attendees will have learned how microscopic groupings of hairs can be used as a basis for sample selection for DNA testing. Examples using both photomicrographs and written descriptions will be presented to assist the listener in understanding how the hairs are sorted into groups. This presentation will demonstrate that evidence hairs, i.e. unknowns, can be intercompared to form groups or sub-sets, that the groups form a valid basis for sample selection for DNA testing, and that such groupings permit sample selection to be based upon potential significance rather than simply whether the hairs are likely to yield results. This offers a way to control for errors introduced by a DNA-only analytical approach.

This presentation will impact the forensic community and/or humanity by demonstrating how incorporating microscopic groupings as a basis for DNA testing of hairs should lead to a reduction in errors introduced into hair evidence by a DNA-only approach and to a reduction in bias inherent in targeted microscopic searches for hairs that may be from a specific suspect among hairs from a victim's person and belongings.

Before DNA testing could be routinely applied to human hair examination, it was not possible to link a hair with a specific individual unless reference samples from that individual were available. Hairs from the arms, legs, eyebrows, eyelashes and so on were not usually suitable for a complete comparison. The conclusions from microscopic hair comparison were not highly specific, and because the quality of work was uneven from laboratory to laboratory and even from examiner to examiner, errors arose that prompted concerns about the reliability of the method. Many examiners reached conclusions from subjective impressions instead of objective data. Adequate training was lengthy and comparisons were time-intensive.

An objective, reliable and highly specific method that could be applied to hairs from any somatic (body area) region became available when it became possible to test hair roots via DNA analysis and use data base searches as an investigative tool if no reference samples were available. However, only hairs with anagen (actively growing) roots could be expected to routinely yield results. Because most evidence hairs are shed rather than forcibly removed, this method could not be successfully applied to most hairs, nor could it be applied to hairs that broke off without the root. This limitation was partly overcome when hair shafts could be tested for mitochondrial DNA, but with new limitations: mitochondrial DNA is not as specific as nuclear DNA, and barring mutations, does not distinguish among relatives in the maternal line, however far removed they may be. Data base searches are not yet possible. Because the hair must be crushed to extract the DNA, any microscopic information is lost during sample preparation, and if the results are inconclusive, microscopic analysis would no longer be possible.

Despite the limitations of microscopic hair comparison, microscopic examination can provide many types of information that DNA testing does not: somatic (body area) origin, the growth stage of the hair root and any putrefaction; adhering debris, and whether the hair itself is likely to be older debris; chemical treatment and mechanical damage; decomposition and insect damage; and so on. These types of information can provide a time line for the hair deposit and assist in determining its significance. The examinations can be performed even if the microscopist does not have extensive training in morphology-based hair comparison. The value of microscopy is well recognized for these types of examinations.

Less recognized is a more urgent analytical problem: the selection of samples for DNA testing. Since it is seldom possible with current technology to test every hair via DNA analysis, selection of adequate samples is crucial. Significant error can arise from sampling, so that even if the DNA results are accurate, any interpretation of significance may be skewed if the basis for selecting hairs is faulty. Unless a skillful microscopic examination is performed first, the current basis for sample selection is the suitability of the hairs for testing. In other words, the testing determines the sample selection instead of the other way around. Testing – of any kind – should be performed on samples selected for their potential value as evidence. If hairs from several different individuals are represented on an item of evidence, the sampling process should ensure that at least one hair from each person be sampled, and that the somatic origin be considered.

The idea of using sub-sets, or groupings, of hair as a basis for selecting samples for DNA testing was presented by one of the authors at a previous AAFS meeting. Actual examples of such groupings using photomicrographs and written descriptions will be presented. It is not necessary to compare each hair directly with each other hair if the descriptions are adequate, although it is useful to intercompare some of the hairs as a cross-check. The sampling error described above can be obviated by performing a microscopic grouping of hairs from an evidence item, using the same morphological features that would be used for a formal microscopic comparison. Instead of comparing the evidence hairs with reference samples, the evidence hairs would be grouped into sets via an intercomparison of unknowns.

Microscopic grouping and testing of a hair from each group would not be necessary if searching for hairs that



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may be from a victim on the clothing or person of a suspect, in which case it would be better to perform a comparison with reference samples from a victim. Only hairs that can link the suspect to the crime would be significant, and unless sets of debris are being compared, in most cases this would be the victim's hairs. However, the common practice of searching among hairs collected from a victim's person and belongings for hairs microscopically similar to those of a suspect introduces a bias that the suspect is the perpetrator. Microscopic grouping of hairs eliminates this bias and provides a more neutral sampling method of testing the evidence hairs.

Reported is the feasibility of training someone with no prior experience in human hair comparison to perform such groupings. A weeklong training class followed by feedback from experienced examiners would allow examiners who are not primarily hair examiners to sort the hairs into groups but not perform final microscopic comparisons. If no experienced hair examiner is on site, the person being trained can use DNA results of groupings performed on in-house proficiency samples for feedback. Training in either case should incorporate the use of DNA results to test whether grouping criteria are valid. It is to be expected that more than one grouping can be attributed to a single individual. However, if a single grouping includes hairs that can be attributed to more than one individual, the grouping criteria should be reevaluated.

Abandonment of traditional microscopic hair comparisons using reference samples is not recommended. However, with a combination of microscopic groupings followed by DNA analysis of one hair from each group, it is no longer necessary to perform complete microscopic comparisons on every case where hairs may be significant evidence. A smaller number of laboratories nationwide that continue to perform rigorous microscopic comparisons should be able to perform this service for other laboratories in those cases where nuclear DNA testing is not feasible, much as is currently done for mitochondrial DNA testing. For example, microscopic hair comparison should be performed in cases where it is important to distinguish among maternally related family members not distinguishable by mitochondrial DNA, and if there is only a single hair in a microscopically determined group, since mitochondrial DNA testing requires destruction of the hair shaft.

In summary, a combination of microscopic grouping and DNA testing of hairs from each group should allow most cases involving hairs to be examined without compromising cases with the errors that a DNA-only approach introduces, yet without using the more time-consuming formal microscopic comparisons. Fewer laboratories should need to train examiners in more rigorous microscopic hair comparisons, and regional centers or other designated laboratories should be able to handle the microscopic comparisons that will still be needed.

### **Forensic Science, Hair, DNA Testing**