Implementing Hematoylin into Casework at the North Carolina State Crime Laboratory

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ABSTRACT: When a hair root is sent for DNA analysis, the hair examiner has determined that this hair may provide valuable information to the investigators. However, sending a hair root for DNA analysis is a destructive test and no further information can be obtained from that root if a DNA profile is not developed. The hair examiners in the NCSC Forensic Trace Evidence Section noticed in years prior to 2019 that hair roots being forwarded to the NCSC Forensic Biology Section for DNA analysis were not yielding profiles as expected. The recent advancements in the Forensic Biology Section’s detection limits prompted the Trace Evidence hair examiners to research ways to improve the current procedure for determining hair root suitability for DNA analysis.

The NCSC Trace Evidence Section noticed in years prior to 2019 that hair roots being forwarded to the NCSC Forensic Biology Section for DNA analysis were not yielding profiles as expected. Therefore, roots monitored from March 2019 to February 2020 were monitored. During this time approximately 1,089 roots, with and without follicular tags, were been stained during casework to ascertain where tissue is located that may contain DNA, the results are marked in red below. Of these 828 root samples, 199 passed the quantification cut-off limit resulting in a 69% overall success rate and a 37% increase in hair roots passing quantification cut-off for DNA analysis. Additionally, Hematoylin root staining revealed 9 roots without follicle tissue passing the nuclei threshold though only one of these roots passed DNA quantification cut-off.

RESULTS

In Phase II of the study, a decrease in DNA quantification pass rate between the Group II Screen for DNA (80%) and Group I (25%) was noted. This decrease was attributed to the implementation of Hematoylin staining coupled with the recent advancements in the Forensic Biology Section’s detection limits prompting the Trace Evidence hair examiners to research ways to improve the current procedure for determining hair root suitability for DNA analysis.

METHODS

Naturally shed hair samples from approximately 15 volunteers were received in the form of both assumed single-source samples (e.g. hairbrush) and known single-source samples (e.g. hair shed during grooming). The submitted samples were examined under a stereomicroscope for the presence of hairs with telogen roots. Over 700 telogen roots were pulled from the original sample set, all of which were then stained according to the following modified root staining protocol:

1. Soak the root in absolute ethanol for 10 minutes.
2. Soak the root in Modified Harris Hematoxylin for 3 minutes.
3. Rinse the root in destained water, followed by absolute ethanol.
4. Place the hair root on a microscope slide and temporarily mount in xylene or xylene substitute*.
5. View the stained root under a transmitted light microscope and observe the presence of any nuclei.
6. Nail nuclei were in general immediately noted in wet or yelkhe umbilicated.*
7. Count the number of nuclei and designate as suitable or not.

It is important to note that after Hematoylin staining has been completed, the hair examiner has no way to recover the DNA from the root. Therefore, roots monitored prior to 2019 are marked in red below. The investigators noted that hair roots being forwarded to the Forensic Biology Section for DNA analysis in casework is being preserved for potential future analysis. All success rates are highlighted below.

PHASE II — VALIDATION STUDY

The quantitative data from the Hair Comparison casework simulation (hairs temporarily mounted in xylene and sent to the casework lab) showed a clear delineation between Groups I and II, where, 80% of Group I passed the quantification cut-off versus 40% of Group II. This delineation indicates an appropriate markets threshold for potential DNA analysis suitability, and is marked in red below.

FURTHER OBSERVATIONS

A dark purple discoloration of the hair shaft was noted, especially in heavily damaged hairs (due to bleaching and chemical processing) and gray hairs, which could affect a hair comparison should the root be returned prior to this comparison.

REFERENCES