Implementing Hematoxylin 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 NCSCL Trace Evidence Section noticed in years prior to 2019 that hair roots being forwarded to the NCSCL 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 begin researching whether changes needed to be made to the current hair root removal protocol to increase the likelihood of developing a DNA profile from a hair root. Trace Evidence hair examiners decided to validate the method of Hematoxylin staining for use in screening roots in the telogen growth phase for DNA analysis. In this study, over 700 head hairs with telogen roots from approximately 15 living donors were stained with Hematoxylin. After DNA analysis, the data showed that **the cut-off for the minimum number of nuclei required in order to obtain a potential DNA profile at the North Carolina State Crime Laboratory is 11 or more nuclei**. The implementation of Hematoxylin staining into casework in March 2019 has resulted in a **37% increase in DNA quantification cut-off pass rate** and a **14% reduction in the number of hair roots forwarded to the Forensic Biology Section for DNA analysis**.

INTRODUCTION

It is well known within the forensic science community that hairs in the telogen growth phase are the most common types of hair found at crime scenes due in part to the periodic shedding of hairs from a person's body.[1] The telogen phase is the mature stage of the growth phase in which the follicle is dormant or resting.[2] Due to the relatively simple nature of telogen hair follicles, they tend to "contain very little quantity of DNA such that most DNA markers cannot be detected."[3] At the North Carolina State Crime Laboratory (NCSCL), telogen hair root suitability for DNA analysis has historically been determined by the presence or absence of tissue around the root in the form of follicular tags. If tissue was present, the hair root and a small portion of the hair shaft would be removed and forwarded to the NCSCL Forensic Biology Section for DNA analysis; a destructive process as it is unknown if the quantity of nuclei needed to generate a DNA profile was present in the tissue. Researching techniques to improve this process suggested two methods of staining the tissue around hair roots to reveal the presence or absence of nuclei.[4,5] These staining techniques coupled with 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 DNA suitability.

This study was comprised of three main phases. Phase I: In July 2017, all available data for hair cases (Screen for DNA and Hair Comparison) completed at the NCSCL over the last nine years was compiled and reviewed. The compiled data included somatic origin, root growth phase (if noted), and whether a DNA profile was generated. The data showed that **50% of all hair roots forwarded to the Forensic Biology Section for DNA analysis over the nine year period were in the telogen phase**. Of these telogen phase hair roots, only **27% yielded a partial or full DNA profile**. Phase II: Based on this data, Hematoxylin staining for use in screening roots in the telogen growth phase for DNA analysis was selected for validation. Phase III: Hematoxylin staining was implemented into NCSCL hair casework in March 2019. The proportion of hair roots passing the Forensic Biology Section's DNA quantitation step was monitored over a period of approximately two and a half years (March 2019 — September 2021) to establish an updated success rate. It should be noted prior to 2017, the Forensic Biology Section had not yet implemented a DNA quantification cut-off. Therefore, data monitored prior to 2017 refers to DNA profile obtained while data monitored following 2017 refers to proportion of hairs passing DNA quantification.

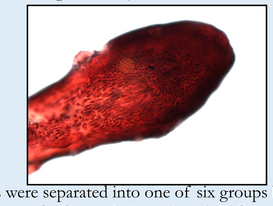
METHODS

Naturally-shed head hair samples from approximately 15 internal volunteers were received in the form of both assumed single-source samples (e.g. hairbrushes) and known single-source samples (e.g. hairs 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[6]:

1. Soak the root in absolute ethanol for 30 minutes.
2. Soak the root in Modified Harris Hematoxylin for 3 minutes.
3. Rinse the root with deionized water, followed by absolute ethanol.
4. Place the hair on a microscope slide and temporarily mount in xylene or xylene substitute*.
5. View the stained hair root under a transmitted light microscope and observe the presence of any nuclei. Nuclei appear dark red or purple in color and typically oval in shape.

**To simulate casework performed at the NCSCL, the hair roots were stained and mounted in water or xylene substitute (Screen for DNA) or a mounting media (Hair Comparisons) following the Trace Evidence technical procedures effective at the time of the study.[7]*

To ensure that the Modified Harris Hematoxylin would perform as expected, a freshly plucked anagen hair root was stained. Numerous dark red or purple ovals indicative of nuclei were noted as shown below (taken at 200x magnification).



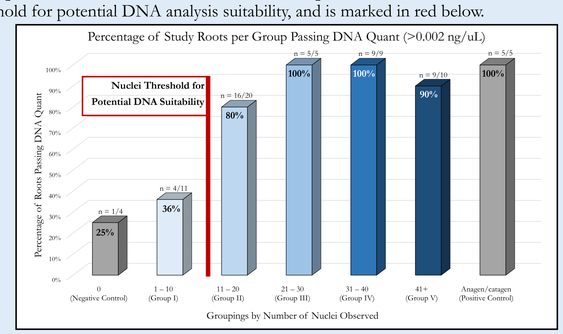
The roots were separated into one of six groups based on the number of nuclei noted; Negative Control (no nuclei noted), Group I (1 to 10 nuclei), Group II (11 to 20 nuclei), Group III (21 to 30 nuclei), Group IV (31 to 40 nuclei), and Group V (41 or greater nuclei). A Positive Control group comprised of anagen and catagen roots derived from the donated sample set was created. A sampling of 64 hair roots, including the Negative Control and the Positive Control Groups, were forwarded for quantitative analysis in the Forensic Biology Section. All samples in the Negative Control Group, Group I, and the Positive Control Group were then amplified along with a representative sample of Groups II, III, IV, and V based on combinations of quantity at or above threshold and degradation index (low and high).

STR DNA analysis was performed on the hair roots using Forensic Biology technical procedures effective at the time of the study.[8]

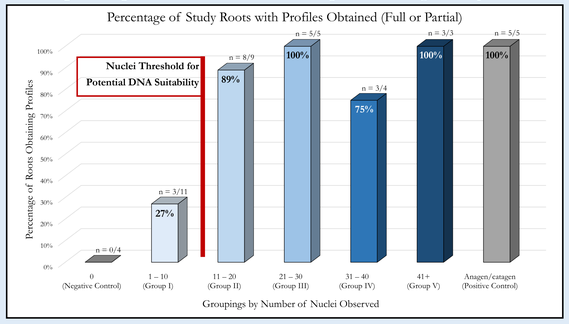
- Qiagen®EZ1 Advanced XL & DNA Investigator Kit
- Qiagen® QIAgility Instrument
- Applied Biosystems™ Quantifiler™ Trio Kit and 7500 Real-Time PCR System
- Promega PowerPlex® Fusion 6C Amplification Kit
- Applied Biosystems™ ProFlex Thermal Cycler
- Applied Biosystems™ 3500 Genetic Analyzer
- Applied Biosystems™ Genemapper™ ID-X software v1.4

PHASE II — VALIDATION STUDY

The quantitative DNA data from the Screen for DNA casework simulation (hairs temporarily mounted in xylene or xylene substitute) showed a clear delineation between Groups I and II, where **36% of Group I passed the quantification cut-off verses 80% of Group II**. This delineation indicates an appropriate nuclei threshold for potential DNA analysis suitability, and is marked in red below.

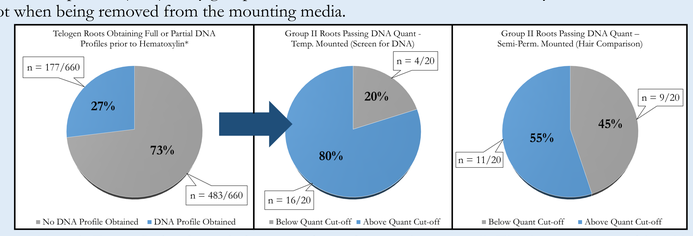


After amplification, the delineation between the results of Groups I and II maintained constant with **27% of Group I obtaining DNA profiles verses 89% of Group II** obtaining DNA profiles, as shown below.



A root must contain 11 or more nuclei in order to be considered suitable for DNA analysis at the NCSCL.

The quantitative data from the Hair Comparison casework simulation (hairs semi-permanently mounted in Permount or Cytoseal) using 20 telogen growth phase hair roots in Group II (11 to 20 nuclei) showed there was a decrease in DNA quantification pass rate between the Group II Screen for DNA (80%) and Group II Hair Comparison (55%) study groups. This decrease could be accounted for by tissue loss from the hair root when being removed from the mounting media.



**Prior to 2017, the Forensic Biology Section had not yet implemented a quantification cut-off. Therefore, roots monitored from 2008-2017 refer to DNA profiles obtained rather than passing DNA quantification.*

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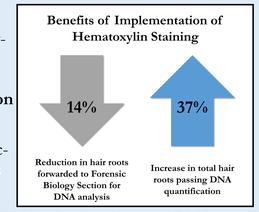
PHASE III — APPLICATION OF THE STUDY TO CASEWORK

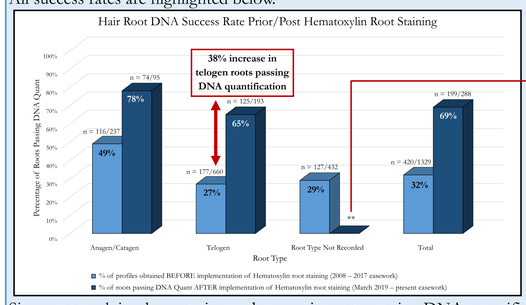
The goal of this study was to increase the success rate of generating a DNA profile from hair roots in casework. Since validating the use of Hematoxylin and implementing the protocol into procedure in March 2019, the application of Hematoxylin staining has been monitored. During this time approximately 1,089 roots, with and without follicular tags, have been stained during casework to ascertain where tissue is located that may contain nuclei and whether all telogen roots need to be stained in the future.

Prior to Hematoxylin, 511 roots with follicular tags would have been forwarded to the Forensic Biology Section for DNA analysis. However, staining determined only 439 of those 511 roots passed the nuclei cut-off with 11 or more nuclei noted. This resulted in a **14% reduction in hair roots being forwarded to the Forensic Biology Section for DNA analysis in casework**.



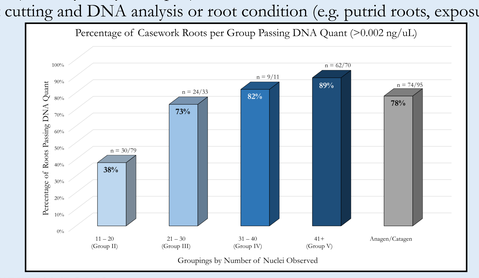
Additionally, Hematoxylin root staining revealed 9 roots without follicular tissue passed the nuclei threshold though only one of these roots passed DNA quantification cut-off.

As certain case scenarios allow multiple roots to be placed in a single DNA tube (e.g. alternate DNA standard), a total of 288 root items were forwarded to the Forensic Biology Section for DNA analysis according to NCSCL Screen for DNA procedure. Of those 288 root items, 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**. Another benefit to Hematoxylin staining is hair roots that are not suitable for DNA analysis at the NCSCL under current procedures are being preserved for potential future analysis. All success rates are highlighted below.



***Case notes from 2008 to 2017 did not always indicate root growth phase for each hair. Hairs without root growth phase noted were placed in this category. Growth phases for all roots were recorded after March 2019.*

Since casework implementation, a decrease in roots passing DNA quantification has been observed near the nuclei threshold (formerly study Group II) as shown below. Potential reasons for this decrease may be time between root cutting and DNA analysis or root condition (e.g. putrid roots, exposure to environment).



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 stained prior to the comparison.
- Staining of degraded and putrid roots revealed dark purple "freckles", smaller in size than the nuclei seen in the freshly pulled roots. Four root items noted as degraded or putrid (37 total anagen and 1 telogen) were forwarded to the Forensic Biology Section for DNA analysis in casework since March 2019. Only one of the anagen roots passed DNA quantification cut-off.
- Of the above-mentioned 9 telogen roots without follicular tissue, only one later passed the DNA quantitation step, indicating a **0.09% success rate**. Therefore, the Trace Evidence Section has determined **analyst discretion is appropriate when considering staining a telogen root with no follicular tag**.

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