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 Trace Evidence Section noticed over the last several years that hair roots being sent for DNA analysis were not yielding DNA 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 has resulted in a 26% increase in quantification cut-off pass rate and a 33% reduction in the number of hair roots sent 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 sent 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 NCSCL 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.

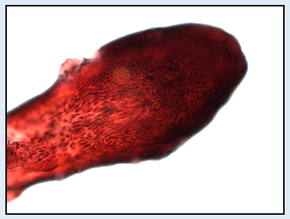
In July 2017, all available data for hair cases (Screen for DNA and Hair Comparison) completed at the NCSCL over the last nine years was compiled and reviewed. The compiled data included somatic origin, root growth phase (if noted), and whether a DNA profile was generated. The data showed that 50% of all hair roots sent 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. Based on this data, hematoxylin staining for use in screening roots in the telogen growth phase for DNA analysis was selected for validation.

METHODS

Hair 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 with a transmitted light microscope and record the presence of any nuclei. The nuclei are dark red or purple in color and usually oval in shape.

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).



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 NCSCL Trace Evidence Hair technical procedures effective at the time of the study.[7] In the first part of this study, over 700 head hairs from approximately 15 living donors were examined for the presence of telogen roots with nuclei. In the second part of the study, 20 hairs with telogen roots were mounted in either Cytoseal or Permount and examined for nuclei. The media was allowed to cure, hairs were then removed from the mounting media, and washed in a xylene-water-ethanol rinsing process.

The roots were separated into one of six groups based on the number of nuclei present; 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), Group V (41 or greater nuclei), and a Positive Control (anagen/catagen). A set of 64 hair roots, including the Negative Control and the Positive Control Groups, were sent for quantitative analysis in the NCSCL 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 NCSCL Forensic Biology technical procedures effective at the time of the studies.[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 AnalyzerApplied Biosystems™ Genemapper™ ID-X software v1.4

RESULTS AND CONCLUSIONS

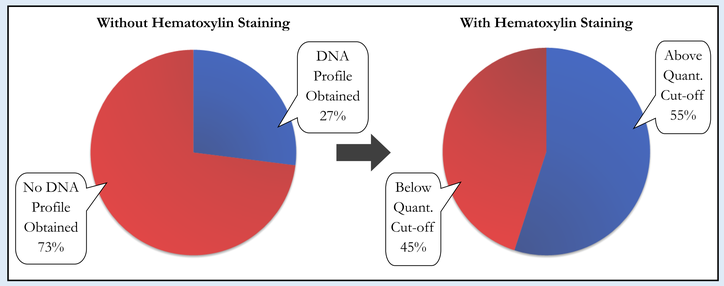
The quantitative data from the Screen for DNA casework simulation showed a clear delineation between Groups I and II, where 36% of Group I passed the quantification cut-off verses 80% of Group II.

Groupings	# of nuclei noted	# of hairs per group	# of hairs that passed quant. (>0.002ng/uL)	% of hairs that passed quant.
Negative Control	0	4	1	25%
Group I	1 – 10	11	4	36%
Group II	11 – 20	20	16	80%
Group III	21 – 30	5	5	100%
Group IV	31 – 40	9	9	100%
Group V	41+	10	9	90%
Positive Control	Anagen/catagen	5	5	100%
Total Hairs Analyzed		64		

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.

Groupings	# of hairs sent for amplification	STR Profiles Obtained			
		Full Profile	Partial Profile	Inconclusive	% of profiles obtained
Negative Control	4	0	0	4	0%
Group I	11	1	2	8	27%
Group II	9	2	6	1	89%
Group III	5	1	4	0	100%
Group IV	4	3	0	1	75%
Group V	3	3	0	0	100%
Positive Control	5	1	4	0	100%

The quantitative data from the Hair Comparison casework simulation using 20 telogen growth phase hair roots in Group II (11 to 20 nuclei) showed that 55% were above the quantification threshold and would have proceeded to DNA amplification. Therefore, a root must contain 11 or more nuclei in order to be considered suitable for DNA analysis at the NCSCL.



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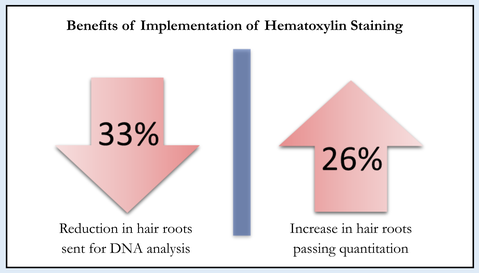
APPLICATION OF THE STUDY TO CASEWORK

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 150 roots, with and without follicular tags, have been stained to ascertain where tissue is located that may contain nuclei and whether all telogen roots need to be stained in the future.

Prior to implementing hematoxylin staining, 36 of the approximately 150 roots would have been sent for DNA analysis. However, staining determined only 24 of those 36 roots passed the nuclei cut-off with 11 or more nuclei noted. Of those 24 roots, 14 passed the quantitative cut-off limit resulting in a 58% success rate. Thus far, implementation of hematoxylin staining has resulted in a 26% increase in quantification cut-off pass rate and a 33% reduction in the number of hair roots sent for DNA analysis. Consequently, hair roots that are not suitable for DNA analysis at the NCSCL under current procedures are being preserved for potential future analysis.

	BEFORE Implementation of Hematoxylin Root Staining (2008 – 2017 casework)			AFTER Implementation of Hematoxylin Root Staining (March 2019 – present casework)		
	# of roots sent for DNA analysis	# of roots that generated DNA profiles	% of profiles obtained	# of roots sent for DNA analysis	# of roots that passed quant.	% of profiles obtained
Anagen	184	99	54%	5	3	60%
Catagen	53	17	32%	1	1	100%
Telogen	660	177	27%	18	10	56%
? root *	432	127	29%	-	-	-
Total	1329	420	32%	24	14	58%

** 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. All roots were monitored for their growth phase after March 2019.*



FURTHER OBSERVATIONS

Observations throughout the study and since implementation of hematoxylin staining into casework:

- 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.
- Staining of degraded and putrid roots revealed dark purple "freckles", smaller in size than the nuclei seen in the freshly pulled roots. Five degraded or putrid roots (three anagen and two telogen) were sent for DNA analysis in casework since March 2019. Only one of the anagen roots passed DNA quantification cut-off but did not have enough alleles for interpretation.
- There was a decrease between the results of the quantitative data of Group II Screen for DNA (80%) and Group II Hair Comparison (55%). This decrease could be accounted for by tissue loss from the hair root when being removed from the mounting media.
- The 30 minute ethanol soak during the hair root staining process, used to stop degradation[6], increased the process time for a Trace Evidence Hair exam. Future studies could test whether a shorter soak time would stop the degradation as effectively.
- Continue monitoring casework to determine whether all telogen roots need to be stained and if there is a correlation between the location of nuclei and the presence of a follicular tag.

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