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An Investigation of the Effect of Temperature and Humidity on the Formation of Antemortem Root Banding

Mae Griffin

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An Investigation of the Effect of Temperature and Humidity on the Formation of Antemortem Root Banding

Mae Griffin

A thesis presented in partial fulfillment of the requirements of the Undergraduate Honors Program at the University of New Haven.

Thesis Advisor: ____________________________
(Signature)

Department Chair: ____________________________
(Signature)

Honors Program Director: ____________________________
(Signature)

May 10, 2022
Date
An Investigation of the Effect of Temperature and Humidity on the Formation of Antemortem Root Banding

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**ABSTRACT**

Microscopical hair analysis has become a controversial practice within forensic science primarily due to issues related to interpretation, and specifically overstating the significance of an association. The validity of microscopical hair comparisons has suffered from intense scrutiny by the forensic community, with one criticism being a paucity of peer reviewed research. An often-unrecognized aspect of microscopical hair analysis is its use in providing reconstructive information based on identifiable features. One example of this is postmortem root band or PMRB. PMRB is the formation of a band slightly above the root bulb and below the skin surface on the hair of cadavers. PMRB can be used as a tool to determine the presence of a cadaver at a crime scene, corroborate witness and suspect testimonies, and create a rough estimate of post mortem interval. The formation of PMRB is still a minimally researched topic with forensic hair analysts slowly investigating the formation of this root band. However, an even smaller amount of research focuses on antemortem root banding (AMRB), which is the formation of a root band in hair that has been sampled from living people. This AMRB is formed from environmental conditions with factors that are poorly understood. In this study, hairs were sampled (pulled) from voluntary individuals and analyzed with microscopic techniques before and after being subjected to various humidity, water, and temperature conditions. This experiment was completed in two phases, with the initial phase centered around the comparison of hairs in three conditions. These conditions were room temperature, body temperature with no added humidity, and a high temperature with high humidity. The second phase of this experiment focused on the comparison of hairs in room temperature conditions, high temperature and high humidity conditions, room
temperature with added water conditions, and room temperature with added soapy water conditions. The results of this study indicate a need for further testing of AMRB formation in different environmental conditions.

Keywords: Trace Evidence, Hair Analysis, Microscopic Hair Examination, Postmortem Root Banding, Antemortem Root Banding, Environmental Effects

INTRODUCTION

Hair analysis is an important field in forensic science, albeit one riddled with issues relating to a need for further research. Forensic hair examiners compare morphological characteristics between questioned and known hairs to come to a conclusion regarding the potential source of the hair. In two such cases, postmortem root banding (PMRB) was key evidence that shaped the outcome of the case [1]. One of these cases involved hairs discovered on duct tape that was speculated to be wrapped around a victim’s head, and another case with the presence of brushy roots and a lack of PMRB. In each of these cases, postmortem changes observed in hair roots were used to match known and unknown hairs, verify witness testimony, and assist in crime scene reconstruction.

Hair itself is a part of the integumentary system, which is located in the scalp, axillary, pubic, facial, nasal, and digital regions [2]. Keratin is the helical protein polymer serving as the makeup of the fibrous material of hair, nails, and skin. Between these proteins are melanin, which are pigment granules, and cortical fusi, which are vacuoles filled with dispersed air or fluid. Hair is composed of 3 layers, the cuticle, the cortex, and the medulla. The cuticle is the external layer of hair made of 6 levels of overlapping,
flattened cells. The cortex accounts for 88% of the hair shaft, formed from elongated, fused, and hardened cells [2]. This layer is where the melanin granules and cortical fusi can be found. The innermost layer, the medulla, is a column of cells running through the center axis of the shaft. The medulla has multiple variations, absent, continuous, discontinuous, or fragmented. However, the most important part of the hair for this research is the root end, which is only visible microscopically. Hair grows from an organ called the hair follicle, which is about 1-4mm below the outermost layer of the epidermis. The anagen phase of hair growth is the most relevant phase of development for this experiment. In the anagen phase, hair is actively growing from the follicle, making up about 80-90% of the scalp. With this continuous growth, hair that is forcibly removed from the scalp can produce a root sheath [2].

PMRB is a distinctive phenomenon which has only been the subject of minimal published research. However, understanding this change can assist in understanding antemortem root banding as well. PMRB is described as “an opaque ellipsoidal band that appears to be composed of a collection of parallel elongated air spaces and is approximately 0.5 mm above the root bulb and about 2 mm below the skin surface” [3]. Other types of postmortem changes in hair can occur, such as decomposition in the proximal end of the hair resulting in incomplete root banding, brush-like cortical fibrils in the proximal end, and a hard-keratinized point in the proximal end. Anagen hair is essential for the study of decomposition of a hair root as degradation only occurs in anagen hair from cadavers [3,4]. PMRB induction has been researched for several factors, most notably being environmental conditions. One such study found that hairs stored in -70 degrees Celsius failed to form PMRB, while hairs sampled prior to
environmental exposure and grown separately from the scalp formed the same result.

Thus, researchers suggested that hair must remain in the anagen follicle inside of the skin for PMRB to form. Hairs that remained within the scalp at room temperature showed banding after twenty-four hours, with banding frequency increasing at 48 hours. Samples at four degrees Celsius never met the banding standards, instead forming minimal banding. [5]. Another study found that over time, PMRB became more evident in frequency with intrinsic variability decreasing. An aqueous environment combined with microbial activity was found to be the cause of the formation of PMRB, in addition to ammonium acetate, suggesting a chemical component as well [6]. The source of hair degradation in PMRB may be caused by protein degradation, as proteomic analysis of PMRB by researchers revealed that non-banded hairs had more protein sequence coverage [7]. However, contrary to what some may assume at first glance, the root of the hair is not entirely damaged. Instead, the degradation in PMRB is located in the cortex of the hair, not in the cuticle [8]. In another study, PMRB was induced with warm weather and vehicle interiors that increased the rate of band formation compared to water, air-conditioning, and cold weather. However, higher temperatures only led to more PMRB changes, as early stages of degradation were present regardless of temperature differences [9]. One study determined that there is a correlation between the frequency of postmortem root degradation and growth stage, hair color, cuticle thickness, cuticle scale profile, and age [10]. PMRB is essential to understand for antemortem root banding due to their similar location and appearance of banding.

Antemortem root banding (AMRB) is a relatively recent discovery in the field of forensic hair examination. AMRB is the formation of a band with a similar appearance to
PMRB [2]. Antemortem root banding is becoming more prevalent as lawyers begin using this limited field of research to defend clients [3]. One such study that examined this phenomenon analyzed the environmental effects on AMRB formation through differing soil and water conditions [2]. With a negative control of indoor air exposure, the researchers tested soil burial and pond water immersion followed by examination with brightfield light microscopy. This study discovered that there was shriveling and erosion of the root structure, banding, or degradation of the root structure beginning at 24 hours and continuing until 4 days of exposure [2]. Antemortem root banding can be split into several categories, consisting of incomplete root banding, root banding, brush-like cortical fibrils, and hard-keratinized point morphologies [3]. Complete root banding was induced in water, saline, outdoor soil, and showers. Brush-like cortical fibrils and hard-keratinized point morphology at the proximal end were only found in the shower. Incomplete root banding at the proximal end was found in water, saline, and showers [3].

**MATERIALS AND METHODS**

Approval to test on human subjects was provided by the University of New Haven Institutional Review Board (IRB) on January 21, 2022. Copies of the IRB forms are in Appendix I. Participants were provided information about PMRB and AMRB, including the definition, the goals of the study, and the requirement for voluntary status. Five individuals were recruited and asked to provide 30-40 hairs from varying regions of the scalp. The IRB stated that hair removal must solely consist of plucking to retrieve Anagen hairs and the retrieved hair must be labeled in a plastic bag. Participants must have been 18 years or older to participate. Participants were also asked to record what type of hair care products they used along with any products applied to the hair. The only
potential risk to participants in this study was minor scalp irritation from the plucking process. The importance of the study was explained so participants can make a decision involving informed consent. Collected samples were also noted to have no individualizing identifiers attached to them and were assigned an anonymous code for sample identification. Participants were notified that their samples could be removed from the project at any time. The researcher in this study also attained certification with the Collaborative Institutional Training Initiative in ethical undergraduate student research procedures. Participants were asked about their chromosomal sex, race, natural hair color, recent hair dyeing or chemical treatments, what shampoo and conditioner is used, any hair care products used, and any illnesses affecting hair or skin. Hairs were provided to the researcher in plastic bags labeled with the time collected and the identifying subject number.

Hairs were retrieved from the plastic bags and analyzed with a stereomicroscope before being subjected to experimental conditions. Hairs were examined for the correct anagen growth stage and orientation of the root end. All hairs were examined and stored in room temperature conditions until the experiment began. Once hairs were initially microscopically examined, they were immediately glued to a microscope slide. Slides were labeled with the examiner’s name, the experiment abbreviation, the date, and the experimental conditions the slide would be exposed. Wet A Hook tech., Tuffleve, Finish/Blue light curing gel was used as an adhesive for the hairs to the slides. A minimal amount of gel was placed on a slide to enable 3 to 5 hairs from a single subject to be adhered to the slide in the middle, leaving both the distal and proximal ends exposed. The gel was then exposed to blue light which cured it into a hard substance that locked the
hairs into place for easier examination. Two slides were made for each subject in each group, leaving a total of 10 slides for each experimental condition.

Phase 1 of the experiment studied the formation of AMRB in three different temperature and humidity conditions (room temperature, body temperature, and high temperature with high humidity) for 4 weeks. The room temperature group was placed into a desk cubby with unfiltered access to the room to ensure that it was safely located in an area where it would still be exposed to the room conditions. The room temperature group was exposed to an average temperature of 22°C and 19.6% relative humidity (RH). The body temperature group was placed in an oven which maintained an average temperature of 33.9°C and 12.5% RH. The high temperature group was placed in an oven which maintained an average temperature of 37.6°C and 26.4% RH. The high temperature was equipped with a Govee Home Temperature Monitor. The high temperature oven was also filled with a water pan which was replaced every Tuesday and Thursday to ensure constant humidity.

All hairs were also examined with a Leica DM EP brightfield light microscope at 100-times Magnification for the first week of Phase 1 on Tuesday and Thursday. This microscope was eventually replaced by the Olympus BX51 brightfield light microscope at 100-times Magnification for the subsequent three weeks of Phase 1 to visualize the root banding on darker hair more clearly. During examination, a single microscope slide was removed one at a time and placed on a paper towel. The hairs would then be mounted with a single drop of Deionized water on the microscope slide, followed by a microscope slide cover being placed over the exposed root end of the hairs. The slide would then be fastened onto the microscope stage, and examination would begin. Kohler
illumination was established prior to all microscopical analysis to ensure best and consistent imaging. Root ends of the hairs were then identified, followed by classification of whether there was banding or no banding. The examiner took a strict approach with classification, identifying bands by their appearance above the root bulb with discontinuation of dark color between the root bulb and the band. Pictures of the hair were taken after determination of root banding with a DinoXcope camera. All subjects were imaged upon initial observations, but only subject 004 was imaged in the following weeks upon the presence of continuous unchanging results. Hairs and slides were dried by patting a Kimtech wipe upon the slide once finishing examination. Samples were then returned to their respective experimental conditions. Upon conclusion of Phase 1, samples were appropriately discarded in preparation for Phase 2.

Phase 2 of the experiment began by renewing voluntary consent from the 5 subjects and re-explaining the new goals of the experiment to re-collect hairs from them. 30 to 40 hairs were collected and placed in plastic bags marked with the time of collection by the subjects. The hairs were then individually gathered from the plastic bags with forceps and examined with a stereomicroscope to verify their anagen growth phase and the orientation of the root bulb. The hairs were then glued to microscope slides labeled with the examiner’s name, the shorthand name for the experiment, the date, and the experimental group and subject. The microscope slides were only used for the room temperature and high temperature groups. The hairs were adhered to the slides in the same manner as Phase 1, using the Wet A Hook tech., Tuffleve, Finish/Blue light curing gel. For the water and soapy water groups, 5 hairs from each subject were placed into 5 individual SHEYNIAN Spice Muslin Bags.
The experimental portion of Phase 2 lasted for a period of 1 month. The experimental groups studied were room temperature, high temperature and high humidity, water at room temperature, and soapy water at room temperature. Humidity and water were replenished every Tuesday and Thursday throughout the experimental period. A water tray was replaced at this rate on the bottom of the oven for the high temperature and high humidity group to maintain constant humidity. The temperature within this oven was monitored with the Govee Home Temperature Monitor. For the water and soapy water groups, the muslin bags were refilled at the rate of twice a week. The water group was filled halfway to full with tap water and allowed to dry off enough to stop leaking. If the bag was not able to be filled halfway, it would instead be watered enough for the entire bag to become wet. The soapy water group was filled with 1 pump of Thermo Scientific Antibacterial Soap, then filled halfway to full and allowed to dry off enough to stop leaking. If the bag was not able to be filled halfway, it would instead be watered enough for the entire bag to become wet. Soap bubbles were allowed to remain within the bag, so long as all of the water was emptied out and the bag was not noticeably soapy to the touch. The water and soapy water groups were placed together into a water pan once dried off after a few minutes. This water pan was left in a room temperature setting on a desk, leaving the samples to dry until their next water exposure. The room temperature slides were placed into a desk cubby with unfiltered access to the room to ensure that it remained protected in a room temperature environment. The room temperature groups had an average temperature of 31.5.0 °C with a 37% RH and the high temperature groups had an average temperature of 37.7 °C with a 53.9 % RH.
At the 2- and 4-week mark of this phase of the experiment, hairs were microscopically examined. The microscope used was an Olympus BX51 brightfield light microscope with a DinoXcope camera for imaging. One photo of potential incomplete root banding was taken with an iPhone XR. Hairs in the room temperature group and high temperature groups were examined by removing the microscope slides one at a time. The slides were placed onto a paper towel and the hairs were mounted with a drop of deionized water. A microscope cover slide was placed onto the hairs, which were then exposed to the Olympus BX51 Light Microscope for examination. Once the status of the banding was determined, images were taken of every sample. The hairs and slide were then patted dry with a Kimtech Wipe. The water and soapy water groups were examined differently by placing a single hair onto a microscope slide without glue. The hair was placed in a manner that allowed for examination of both ends under the microscope. The hair was mounted with deionized water and a cover slip was placed over both ends of the hair. The sample was then analyzed under the Olympus BX51 brightfield light microscope with subsequent imaging. The slides were patted dry with a Kimtech Wipe and the hair was returned to the bag. All samples were returned to their respective experimental conditions upon conclusion of data collection at week two. Note: upon replacement of the water for the high temperature oven on Tuesday May 3, 2022, the temperature monitors within the oven were discovered to be melted. The high temperature experimental conditions were discontinued after this, as the temperature was adjusted by an unknown external source. As such, only the water and soapy water samples were studied at the end of Phase 2. After the conclusion of data collection in week 4, samples were appropriately discarded.
RESULTS AND DISCUSSION

No complete banding was recorded in Phases 1 and 2 of the experiment. Photomicrographs were taken of hair from each subject within each experimental group on the first day of data collection in each phase. Subsequent photo-documentation focused on a single subject (004), as the hair was considered the easiest to examine due to its light color. The presence of root banding was determined through quantitative and qualitative means. Root banding occurs about 0.5 mm above the root bulb [3]. The issue with this quantitative means is that the ending of the root bulb is unclear in certain hairs. There is no clear line between where the root bulb ends and where the shaft begins. Examiners must approximate the ending of the root bulb, leading to some potential inaccuracies with this method. Instead, a strict qualitative assessment served as the primary means of identifying root banding. Banding was determined to require a clean break in color between the root bulb and the start of the band as it appeared closest to the bulb. Banding must also interrupt the features of the hair with its black discoloration, such as by obscuring the medulla should it be visible. This standard was established based on images taken of antemortem root banding in previous experiments where the majority of banding featured a dark band disconnected from the root bulb [2,3]. This was the major assessment method used to disqualify certain discolorations from identification as root banding.

Figure 1 displays a photomicrograph of a hair from subject 004 showing no banding after 28 days of exposure to room temperature conditions. The coloration seen in the hair is not indicative of banding because there is no clear separation from the root bulb nor other features indicative of root banding. Instead, this discoloration was found
throughout a majority of the samples around the bend of the root bulb. This discoloration was found in the samples from before the experiment began and was identified as a trait that was common to the subject's hair in this research. This coloration was also more clearly visible when examined through the microscope eyepieces than what was captured in the photomicrographs. This may have resulted from the quality of the camera which resulted in the exaggeration of the darker color. Figure 2 depicts an photomicrograph of a hair from subject 005 showing no banding after 28 days of exposure to water conditions. The cortex can be seen running through the center of the hair continuously with the surface texture features of the hair less visible. No banding was depicted here as this photomicrograph shows a clearer version of the discoloration at the root bulb’s bend instead. If a band were to be present, the discoloration would still be present in addition to another discoloration further along the shaft. Figure 3 depicts this as well, showing how the discoloration noted can extend across the bulb entirely. If a band were to be present here, it would display closer towards the bend as the hair meets the shaft rather than on the bulb as the discoloration currently shows. Figure 4 depicts a discoloration that does not extend to the rest of the root bulb, remaining just above the tip instead. The hair was damaged with signs of breakage noted. This was not considered root banding as the discoloration did not extend towards the shaft and remained isolated around the breaking area of the hair. The hair breakage was most likely an occurrence related to the passage of time or through damage incurred by forceps.
Figure 1: An image of a hair root from subject 004 showing no banding after 28 days of exposure to room temperature, taken at 100-times magnification.

Figure 2: An image of a hair root from subject 005 showing no banding after 28 days of exposure to tap water, taken at 100-times magnification.
Figure 3: An image from a hair root from subject 003 showed no banding after 28 days of exposure to room temperature, taken at 100-times magnification.

Figure 4: An image from a hair root from subject 004 showed no banding after 28 days of exposure to room temperature, taken at 100-times magnification.
Figure 5 displays a photomicrograph of a hair with a dark discoloration that could be misidentified as a root band. However, the location and size of the banding, as well as a lack of parallel elongated air spaces which are characteristic of AMRB, indicate that this is not AMRB [2,3]. This image was taken with an iPhone XR as the DinoXcope camera was unable to capture the contrast between the discoloration and the hair’s natural color. The sample also has a bent root bulb, making measurements from the tip of the bulb to the band much more difficult. In previous experiments, a majority of samples featured a disconnect between the discoloration of the root bulb and the discoloration found closer to the shaft of the hair [2,3]. Some samples did not feature this clean break in color, but the boundaries for determining what constitutes an antemortem root band are still murky as this is a relatively new research topic [3]. Ultimately it was determined that the source of this banding is unidentified, and may be a general discoloration unrelated to antemortem root banding.
Figure 5: An image from a hair root from subject 003 showed no banding after 28 days of exposure to tap water, taken at 100-times magnification.
Figure 6: An image from a hair root from subject 004 showed no banding after 28 days of exposure to body temperature, taken at 100-times magnification.

Figure 7: An image from a hair root from subject 004 showed no banding after 28 days of exposure to room temperature, taken at 100-times magnification.
Figure 8: An image from a hair root from subject 004 showed no banding after 28 days of exposure to room temperature, taken at 100-times magnification

Figure 9: An image from a hair root from subject 004 showed no banding after 28 days of exposure to room temperature, taken at 100-times magnification

Ultimately, no AMRB was able to be successfully created in any of our experimental conditions. The failure to induce root banding may be due to several conditions. These conditions are not definite causes of root banding and are instead proposed causes that may potentially induce root banding. One worth exploring in the future research is a microbial source of banding rather than temperature. Several microbes may be present in hair samples that exhibit root banding. This experiment did not test for bacteria, fungi, or viruses. Other microscopic specimens may also influence banding, but this experiment was conducted in a laboratory as compared to outdoor or common interior settings. The sterile nature of the laboratory may have prevented
contamination of the samples and banding in turn if these microscopic specimens are a source of antemortem root banding. Another variable is chemical components that may damage the hair to induce the banding. For example, certain shampoos may contain substances that can induce banding with chemical degradation should antemortem root banding be caused by such. Finally, banding could be caused by a genetic component. The reason some examiners may be able to induce antemortem root banding while others cannot may be linked to a gene that causes the banding to incur. These variables are not the stated cause of antemortem root banding and serve as a potential cause that is worth investigating in future experiments.

This experiment had some limitations in it. The reliance on the subjective determination by the examiner and limited information regarding PMRB and AMRB meant that analysis was challenging to complete with confidence by a student researcher. The DinoXcope camera also faced difficulties in use sometimes as picture clarity and contrast were highly reliant on the examiner’s expertise with the camera and external light. Figure 7 shows one such image where the hair could not be brought into focus in all parts of the image. Figure 8 shows how subjectivity, errors, and examiner expertise plays a role in interpretations. An inexperienced examiner might initially assume that this might be banding, but the darker appearance of the hair is a result of the sharpie that was used to label the slide. Figures 9 and 10 display hair that could be interpreted as having root banding, but the discoloration found was present on the hairs before experimentation even began.

While this experiment had negative results because no AMRB was able to be induced in our laboratory conditions, it does not mean that this is not valuable
information for the forensic science community. This research provides insight into the
difficulty of inducing AMRB. AMRB requires further research to establish more sources
of the banding, but the large lack of results in the temperature and humidity environments
used in this study can provide insight for designing future experiments. This research was
limited by several factors, such as number of subjects and hairs examined, the time period
of the study, and the microscope camera capabilities, but the ultimate goal of examining
whether there is a minimum temperature and humidity required for inducing AMRB was
accomplished. Although this minimum was not established because no AMRB was
formed in any of the hundreds of examined hairs, this research demonstrates the difficulty
in inducing AMRB.

CONCLUSIONS

The inability to induce AMRB in any environmental condition studied indicates
that the formation of AMRB requires additional research and investigation. Further
experiments should utilize longer timeframes when investigating the effect of
temperature and humidity on AMRB formation. For example, the band in figure 5 did not
have characteristics of AMRB after 28 days, but could have formed into something
darker, longer, or more defined had it been given more time to develop. Further research
must also focus on identifying defined quantitative and qualitative characteristics for the
clear identification of AMRB. The goal of this branch of research would be to
standardize methods of identification to assist other experiments with clear definitions.
Finally, antemortem root banding still requires a large array of research into its formation
and the effects of certain factors, such as exposure to bacteria or various chemical
products. The effects of this banding must also be discovered regarding the hair’s structure to uncover the mechanism behind this type of banding.

AMRB is a relatively new, complex, and highly misunderstood topic in the field of forensic hair examination. There is real need for foundational research to be conducted on AMRB so that it can be understood, especially with regards to differentiating it from PMRB. Should an antemortem root band be found and mistakenly classified as a postmortem root band, this error could close investigative leads that were previously open. Such an error could have impacts that have not been predicted yet on both the defense and prosecution. For the benefit of cases where a hair containing root banding may be identified, the differentiation between antemortem and postmortem root banding is critical for performing a complete and appropriate interpretation and reconstruction.

ACKNOWLEDGEMENTS

I would like to thank Dr. Brooke W. Kammrath for mentoring me in this research. I would also like to thank my family, friends, and loved ones for supporting me throughout my academic career.
REFERENCES


Appendix I

University of New Haven
IRB Disposition Form

Date: January 21, 2022

To: Dr. Ambers

From: Dr. Alexandria Guzmán, IRB Chair

Proposal Title: An Investigation of the Effects of Temperature and Humidity on the Formation of Antemortem Root Banding

Protocol Number: 2021-094

Review by: Committee ________ Date of Meeting ________ Expedited Procedure ________ AEG ________

The IRB has approved the proposed use of human participants in this project.

____ XX _____ The proposal is approved as submitted.

____ The proposal is approved with the following minor stipulations. If you agree to the condition(s) of approval, please sign one copy and return it to the IRB chair. If the condition(s) of approval are not clear or are unacceptable to you, please contact the IRB chair.

____ The proposal has not been approved in its current form. The committee advises that the revisions below be made and the application be resubmitted for further IRB review. If the suggestions for resubmission are not clear or are unacceptable to you, please contact the IRB chair.

____ The proposal has not been approved. Reasons for this decision are provided below.

Project Expires on
JAN 01 2023
University of New Haven
Institutional Review Board
PLEASE NOTE

1. Approval of the project will expire on 1/1/2023. Federal regulations (DHHS) and UNH policy require that you submit a “Continuing Review Form” at that time if continued approval is desired. A copy of the “Continuing Review Form” is attached.

2. If the research procedures are altered from the description in the proposal reviewed, you must submit a “Request for Revision Form” to the IRB.

3. Upon approval of the study, a consent document will be stamped with an expiration date. Only this document may be used when enrolling subjects. Studies extending beyond the expiration date must be submitted for a continuation review. Any changes in the consent form must be approved by the IRB.

4. For projects with multiple data collection points, informed consent must be obtained at each data collection session.

5. When participant recruitment and data collection are completed for this project, federal regulation and UNH policy require that you submit a “Research Completion Form” to the IRB. After this form is submitted, if you wish to recruit more participants or collect more data for this project, you must submit a new IRB Application form for review.

6. Should any modifications be made in the approved project, a “Request for Revision” form must be submitted to the IRB.

7. If any adverse event or data breach occurs during participant recruitment or data collection an “Adverse Event/Data Breach” form must be submitted to the IRB.

8. If any problems arise concerning the welfare of subjects in the projects, please contact me (IRB@newhaven.edu).

It is the responsibility of the Principal Investigator to ensure that any person that joins the research team after initial IRB approval be certified prior to interacting or intervening with human participants or their data.

***If the interviews can be done with appropriate social distancing protocols, then that would be fine. The faculty need to submit the attached COVID-19 Safety Plan for this research and to submit the Campus Access Request Forms so that we have a log of your plans to resume research with students, even if it is at remote locations. If the work will be done on campus, then we would need to know where this work will take place so we can evaluate if we need to bring any additional buildings back online.***

Best wishes for the successful completion of your research.
Informed Consent Form

(University of New Haven)

Title of Project: An Investigation of the Effect of Temperature and Humidity on the Formation of Antemortem Root Banding

Principal Investigator: Brooke W. Kammrath, Ph. D.

Co-Principal Investigator: Angie Ambers, Ph. D.

Co-Principal Investigator: Mae Griffin

Participant's Printed Name:

We invite you to take part in a research study "An Investigation of the Effect of Temperature and Humidity on the Formation of Antemortem Root Banding" at the University of New Haven. This research seeks to answer the question of whether there is a minimum temperature or humidity requirement for the formation of antemortem root banding (AMRB). AMRB is the formation of a band based on certain environmental factors with a similar appearance to postmortem root banding (PMRB), which forms solely on cadavers. Taking part in this study is entirely voluntary. We urge you to discuss any questions about this study with the investigators. Talk to your family and friends about it and take your time to make your decision. If you decide to participate you must sign this form to show that you want to take part.

This research study is being done in order to discover the environmental factors responsible for the formation of AMRB. This study seeks participants varying in age (18 years and above) and biological sex. This study will require you to donate approximately fifty (50) hairs from around your head. The hair will need to be plucked. You will collect your hair in a pre-labeled plastic bag that has been provided by the investigator. The participants will also be asked to record what type of hair care products they use along with any products applied to the hair. The only potential risk to participants in this study is minor scalp irritation from the plucking process. This study has the potential to benefit society by discovering the conditions that form AMRB to help differentiate between AMRB and PMRB. This will help in cases where the life of the victim is called into question.

Collected samples will have no individualizing identifiers attached to them, but will be assigned an anonymous code by which the sample will be identified throughout the course of the study and in any future projects, presentations, and/or publications. The samples will only be used for research purposes. Samples shall be removed from the project and destroyed at any time upon request. At the end of the study and related projects, all samples will be destroyed.

Project Expires on

JAN 9 1 2023

University of New Haven
Institutional Review Board
Participants will receive no compensation for donating samples to this study. This study is funded by the Department of Forensic Science and Honors Department at the University of New Haven.

Taking part in this research study is voluntary. You do not have to participate in this research. If you choose to take part, you have the right to stop at any time. If you decide not to participate or if you decide to stop taking part in the research at a later date, there will be no penalty.

Before making the decision regarding participation in this research you should have:

· Discussed this study with an investigator,
· Reviewed the information in this form, and
· Had the opportunity to ask any questions you may have.

Your signature below means that you have received this information, have asked the questions you currently have about the research and those questions have been answered. You will receive a copy of the signed and dated form to keep for future reference.

Participants: By signing this consent form, you indicate that you are voluntarily choosing to take part in this research, and at least 18 years of age.

Signature of Participant Date Printed Name

If you have any questions or concerns regarding this study or your rights as a research participant and would like to talk to someone other than the researcher(s), contact the chair of the Institutional Review Board at UNH at irb@newhaven.edu

Person Explaining the Research: Your signature below means that you have explained the research to the participant/participant representative and have answered any questions they have about the research.

Signature of Person who explained this research Date Printed Name

Project Expires on JAN 01 2023
University of New Haven Institutional Review Board
An Investigation of the Effect of Temperature and Humidity on the Formation of Antemortem Root Banding

Participant Questionnaire

Participants Number (assigned by the investigator): ________________

1. Select your Chromosomal Sex: Male (XY) Female (XX)

2. What is your racial origin(s) (see categories below): ________________
   a. White – A person having origins in any of the original peoples of Europe, the Middle East, or North Africa.
   b. Black or African American – A person having origins in any of the Black racial groups of Africa.
   c. American Indian or Alaska Native – A person having origins in any of the original peoples of North and South America (including Central America) and who maintains tribal affiliation or community attachment.
   d. Asian – A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam.
   e. Native Hawaiian or Other Pacific Islander – A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.

3. Indicate your Natural Hair Color: ________________

4. In the last 12 months, have you dyed or used chemical treatments on your hair? Yes/No
   a. If yes, when was the most recent treatment? ________________
   b. If yes, what was the treatment/dye? ________________

5. What Shampoo and/or Conditioner do you use?
   ___________________________________________________________________

6. What other hair care products do you use?
   ___________________________________________________________________
   ___________________________________________________________________

7. Do you have any illnesses that affect your hair or skin? Please list them below (Ex: alopecia).
By signing this document, you agree that all information is true.

<table>
<thead>
<tr>
<th>Signature of Participant</th>
<th>Date</th>
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<tr>
<th>Signature of Investigator</th>
<th>Date</th>
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Mae Griffin

Expedited IRB Review Questions

1. The purpose of this experiment is to determine the effect of humidity and temperature on the progression of antemortem root banding (AMRB) with the goal of discovering if there is a minimum humidity and temperature, or combination of the two, required for band formation. Antemortem root banding is the formation of a root band in hair that has been sampled from living people. This AMRB is formed from environmental conditions with factors that are poorly understood. Hair samples will be documented with microscopic analysis before being subjected to varying humidities and temperatures. Humidity and temperature will be controlled by using an oven combined with a humidity chamber using known salts. Samples will be analyzed over several days to weeks with observations made periodically (i.e., days 1, 3, 5, 7, 10, and 14) to determine if antemortem root banding has occurred. The threshold will then be determined based on the time of formation and degree of banding of the AMRB. Samples will be compared to post mortem root banding (PMRB), a band that is similar to AMRB that forms on hairs samples from cadavers instead. These samples may be procured from Dr. Ambers depending on availability from a body farm.

2. Hair samples will be gathered from voluntary living individuals with a sample size of approximately 5 donors with 50 hairs for each donor under each condition. Hairs will be retrieved from UNH staff, faculty, and students who have completed the attached survey and consent form. Restrictions will be made on dyed or treated hair, drug use, or known diseases like alopecia and skin conditions. Samples will not be retrieved from children, prisoners, pregnant women, the mentally disabled, and other protected populations.

3. Hairs will be retrieved from the subjects through plucking. Volunteers will be asked to pluck 50 of their own hairs from all regions around their heads. Hairs will then be placed into a pre-labeled plastic bag provided by the investigator. Participants will be selected after they fill out a participant questionnaire and sign the informed consent form.

4. The only potential risk to participants in this study is minor scalp irritation from the plucking process.

5. Collected samples will have no individualizing identifiers attached to them, but will be assigned an anonymous code by which the sample will be identified throughout the course of the study and in any future projects, presentations, and/or publications. The samples will only be used for research purposes. Samples shall be removed from the project and destroyed at any time upon request. At the end of the study and related projects, all samples will be destroyed.
INSTITUTIONAL REVIEW BOARD
APPLICATION FOR APPROVAL TO USE HUMAN SUBJECTS IN RESEARCH
EXPEDITED/FULL REVIEW

1. Project Title: An Investigation of the Effect of Temperature and Humidity on the Formation of Antemortem Root Banding

2. Principal Investigator (Must be a faculty/staff member at UNH; if thesis project, faculty advisor is PI and student is co-PI):
   Dr. Brooke W. Kammrath

Degree (PhD, MA, MFA, etc...): Ph.D.

Administrative Unit/Department: Forensic Science

Phone (UNH Extension): 203-931-2989

E-mail: bkammrath@newhaven.edu

Status (Mark one):
   X UNH Faculty
   UNH Staff
   UNH Administration

3. Co-PI: Dr. Angie Ambers

Affiliation (If non-UNH) N/A

Affiliation Address (If non-UNH) N/A

Degree (PhD, MA, MFA, etc...): Ph.D.

Administrative Unit/Department: Forensic Science

Phone (UNH Extension): 203-479-4581
E-mail: sambers@newhaven.edu

Status (Mark one):

X UNH Faculty

UNH Staff

UNH Administration

Graduate Student (must have a UNH faculty research advisor)

Undergraduate Student (must have a UNH faculty research advisor)

Other (please explain)

(Non-UNH must have UNH faculty/staff/administrator primary PI)

Co-PI: Mae Griffin

Affiliation (if non-UNH) N/A

Affiliation Address (if non-UNH) N/A

Degree (PhD, MA, MFA, etc...): B.S.

Administrative Unit/Department: Forensic Science

Phone (UNH Extension): N/A

E-mail: mgriff@unh.newhaven.edu

Status (Mark one):

UNH Faculty

UNH Staff

UNH Administration

Graduate Student (must have a UNH faculty research advisor)

X Undergraduate Student (must have a UNH faculty research advisor)

Other (please explain)

(Non-UNH must have UNH faculty/staff/administrator primary PI)

4. Project Status (Mark one):

Faculty Scholarship Graduate Research

Undergraduate Faculty-Mentored Research

Master's Thesis Dissertation Research

Honor's Thesis X Senior Project

SURF Project Other (please explain)
5. For student researchers only, who is/are your UNH affiliated faculty advisor(s)?

Faculty Advisor(s): Dr. Brooke W. Kamrath and Dr. Angie Amberg

Degree (PhD, MA, MFA, etc.): Ph.D.

If different from PI above please provide the following information:

Administrative Unit/Department: ________________________________

Phone (UNH Extension): __________

E-mail: ________________________________________________

Status (Mark one):

_____ UNH Faculty

_____ UNH Staff

_____ UNH Administration

_____ Graduate Student (must have a UNH faculty research advisor)

_____ Undergraduate Student (must have a UNH faculty research advisor)

_____ Other (please explain)

(Non-UNH must have UNH faculty/staff/administrator primary PI)

NOTE: The IRB will not review protocols submitted by students without the signature of a faculty advisor on the signature page.

6. Does your study involve the collection of data from a vulnerable population?

   yes _____ no _____ X____

   If yes, please specify: _____________________________________________

7. Does this study involve deception (research in which the subject is purposely lead to have false beliefs or assumptions)?

   yes _____ no _____ X____

8. If the study involves risk to subjects, is the risk greater than that incurred in ordinary life or tasks?

   yes _____ no _____ X____

9. Has this study ever been previously approved by the UNH Human Subjects IRB?

   yes _____ no _____ X____

10. Has this study ever been previously approved by a non-UNH Human Subjects IRB?

    yes _____ no _____ X____

    If yes, please specify what IRB: _________________________________
If yes, please append the decision of the other IRB to this application.

11. Check if this proposal is ___X___ new or _____ revised in response to previous IRB review.

12. Is funding being sought for this study? If yes, through what sponsoring agency?

Yes, the UNH Honors Department and Forensic Science Department.